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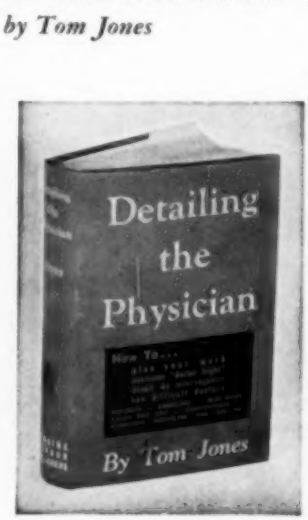

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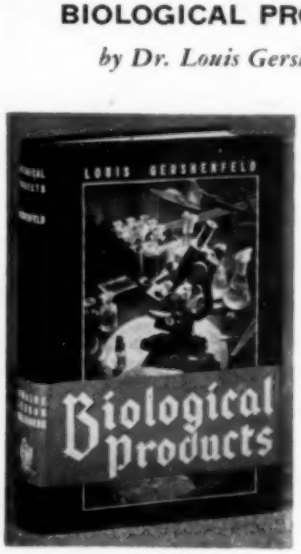

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Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

J. L. Powers, Editor, Washington, D. C.

Volume XXXI *JANUARY, 1942* Number 1
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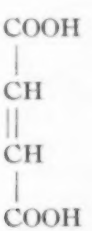
The Toxicity and Laxative Action of Sodium Fumarate*

By Oscar Bodansky, Harry Gold and William Zahm

With the technical assistance of Helen Civin and Charles Salzman

The salts of several carboxylic acids, such as tartaric, citric and gluconic acid, are known to possess laxative properties, and some of them are strongly nephropathic. The present report is concerned with a study of the toxicity and the laxative properties of sodium fumarate.

Fumaric acid is a dicarboxylic acid with the formula:



It was used in the form of the disodium fumarate.

EXPERIMENTAL

ANIMAL EXPERIMENTS

Rabbits and cats were used in these experiments. Several types of observations were made to study toxicity. The behavior, the appearance and the weight of the animals were recorded. Blood examinations were made for evidence of non-protein nitrogen (N. P. N.) (1) and creatinine (2) retention. The jugular vein was exposed through a small incision of the skin. This greatly facilitated the collection of blood samples. The wound showed little tendency to infection. Phenolsulfonephthalein (P. S. P.) excretion tests were made (3). The urine was examined for albumin, casts, reducing substances and red blood cells. These observations were made prior to, and for varying numbers of days after, the administration of the drug. The final data included approximately 90 of each of the foregoing examinations.

At autopsy, the organs were inspected grossly, and in the case of the liver and kidney, microscopic sections were also examined (in all, 44 sections). In animals that were sacrificed, the tissues were fixed immediately in 15 per cent formalin solution. Paraffin sections were made and stained with hemotoxylin and eosin. Postmortem changes complicated the histological examination on those cases in which the animal died during the night.

All doses were given by intravenous injection in 10 or 15 per cent solution in water. This route of administration provided the more severe test of the systemic toxicity of the drug.

Single Dose in Rabbits.—The effect of a single intravenous injection of disodium fumarate was studied in one group of 9 rabbits. The essential data are summarized in Table I. The doses varied from 0.05 to 2.0 Gm. per Kg. of body weight. Anticipating that severe poisoning by the fumarate might result in anorexia, we first planned this group of experiments in fasting animals.

* From the Department of Pharmacology, Cornell University Medical College, and the Medical Service of the Hospital for Joint Diseases, New York.

The drug was supplied by Chas. Pfizer & Co., Inc., to whom we are indebted for a grant which aided this investigation.

Table I.—The Effect of a Single Intravenous Dose of Sodium Fumarate, Sodium Tartrate and Sodium Chloride

Animal No. Initial and final weight (Kg.)	Dose, Gm./Kg.	N. P. N.	Creatinine	P. S. P.	Liver	Histology Kidneys	Remarks
			Rabbits (Fumarate)				
1	0	52 (0)[a]	2.1 (0)	80 (0)	Normal	Cloudy swelling in tubules[b]	Died after fasting 9 days
I. W. 2.51		...	...	65 (1)			
F. W. 1.84		48 (2)	...	100 (2)			
		59 (4)	2.1 (4)	90 (4)			
		60 (6)	1.8 (6)	88 (6)			
		83 (8)	2.1 (8)	68 (8)			
2	0	...	...	100 (0)	Post-mortem degeneration	Post-mortem degeneration[b]	Died after fasting 11 days
I. W. 2.26		72 (2)	2.4 (2)	95 (2)			
F. W. 1.34		91 (3)	2.4 (3)	...			
		74 (4)	2.5 (4)	71 (4)			
		90 (6)	2.5 (6)	75 (6)			
3	0.05	53 (−2)	1.5 (−2)	45 (−2)	...	...	See Table II
I. W. 2.62		55 (−1)	1.1 (−1)	72 (−1)			
F. W. 1.83		...	...	85 (0)			
		59 (+2)	1.4 (+2)	90 (+2)			
		58 (+4)	1.5 (+4)	88 (+4)			
		55 (+6)	1.4 (+5)	74 (+6)			
4	0.20	48 (−2)	1.8 (−2)	90 (−2)	...	...	See Table II
I. W. 2.50		48 (−1)	1.8 (−1)	70 (−1)			
F. W. 1.8		...	...	80 (0)			
		50 (+2)	2.4 (+2)	90 (+2)			
		46 (+4)	2.4 (+4)	78 (+4)			
		51 (+6)	2.4 (+6)	91 (+6)			
5[c]	0.50	37 (0)	1.6 (0)	98 (0)	Normal	Slight cloudy swelling; weight, 10 Gm.[b]	Died 3 days after dose
I. W. 2.83		35 (+1)	1.6 (+1)	84 (+1)			
F. W. 2.80							
6[c]	0.50	...	...	...	...	...	Died of pulmonary edema 10 min. after rapid injection
I. W. 3.17							
7	0.60	52 (−2)	2.2 (−2)	50 (−2)	Normal	Normal[b]	Was found depressed and week 2 days after dose. Died 1 hr. 18 min. after last blood sample, and after fasting 5 days
I. W. 2.70		52 (−1)	2.7 (−1)	45 (−1)			
F. W. 1.97		...	...	50 (0)			
		168 (+2)	5.0 (+2)	...			
8	0.60	54 (−1)	1.7 (−1)	95 (−1)	Normal	Tubules show marked granular swelling[b]	Died after fasting 6 days
I. W. 2.32		62 (0)	1.7 (0)	100 (0)			
F. W. 1.87		61 (+1)	1.7 (+1)	90 (+1)			
		102 (+3)	1.8 (+3)	90 (+3)			
9	1.00	55 (−1)	2.0 (−1)	95 (−1)	Normal	Tubules show marked granular swelling	Died after fasting 7 days; in clonic convulsions for 20 min.
I. W. 2.12		54 (0)	1.9 (0)	100 (0)			
F. W. 1.82		53 (+1)	1.7 (+1)	93 (+1)			
		72 (+3)	2.5 (+3)	92 (+3)			
10[c]	1.00	46 (0)	1.7 (0)	87 (0)	Normal	Normal; weight, 17 Gm.	Died of clonic convulsions 11 days after dose. Brain section also normal
I. W. 2.26		53 (+1)	1.5 (+1)	93 (+1)			
F. W. 2.28		52 (+3)	1.6 (+3)	79 (+3)			
		36 (+5)	1.4 (+5)	64 (+5)			
		44 (+8)	1.2 (+8)	91 (+8)			
		41 (+10)	1.0 (+10)	99 (+10)			
11[c]	2.00	...	...	...	...	...	Died of pulmonary edema 10 min. after injection
I. W. 2.96							
			Rabbits (Tartrate)[d]				
12[c]	0.20	46 (0)	1.3 (0)	110 (0)	Post-mortem degeneration	Granular swelling of tubules. Weight, 27 Gm.[b]	Died 5 days after injection
I. W. 3.14		81 (+1)	2.4 (+1)	58 (+1)			
F. W. 2.70		69 (+2)	2.0 (+2)	53 (+2)			
13[c]	0.30	49 (0)	1.6 (0)	93 (0)	Normal	Normal; weight 25 Gm.	Died 8 hrs. after injection, in convulsions. No gross pathological findings
I. W. 2.78							
F. W. 2.78							

Animal No. Initial and final weight (Kg.)	Dose, Gm./ Kg.	N. P. N.	Creatinine	P. S. P.	Liver	Histology Kidneys	Remarks
14	0.30	44 (−1)	2.0 (−1)	75 (−1)	Normal	Different from fumarates. Marked tubular swelling with Ca deposits. Nephrosis. Weight, 38 Gm.[b]	Died after fasting 7 days
I. W. 2.96		45 (0)	2.1 (0)	91 (0)			
F. W. 2.78		88 (+1)	6.8 (+1)	1 (+1)			
			Rabbit (Sodium Chloride)				
15	0.20	43 (1)	1.8 (1)	100 (1)	...	Weight, 14.5 Gm.	Died on 2nd day, in clonic convulsions for 3 min.
I. W. 2.45							
F. W. 2.45							
1[c]			*Cats (Fumarate)*				
I. W. 2.73	0.00	49 (0)	2.2 (0)	40 (0)	...	...	Used for tartrate injection, and designated cat 1*A* (see below)
F. W. 2.98		70 (1)	2.0 (1)	69 (1)			
		42 (3)	1.7 (3)	54 (3)			
		44 (5)	1.7 (5)	50 (5)			
		34 (8)	1.7 (8)	51 (8)			
		44 (10)	1.5 (10)	66 (10)			
2[c]	1.00	51 (0)	1.8 (0)	66 (0)	Normal	Normal; weight, 22 Gm.	Immediately after injection, respiration became rapid and animal depressed. Sacrificed 25 days after injection
I. W. 3.00		48 (+1)	1.5 (+1)	91 (+1)			
F. W. 3.15		32 (+3)	1.4 (+3)	73 (+3)			
		25 (+5)	1.2 (+5)	61 (+5)			
		30 (+8)	1.4 (+8)	75 (+8)			
		36 (+10)	1.3 (+10)	76 (+10)			
		47 (+25)	1.3 (+25)	65 (+25)			
			Cat (Tartrate)[d]				
1*A*[c]	0.30	44 (0)	1.5 (0)	66 (0)	Fatty infiltration	Large scar (about 2 cm. long) in both kidneys noted grossly although section examined was normal. Weight, 25.5 Gm.	2+ albumen and 4+ glucose in urine 4 days after injection. 4+ glucose 7 days after injection. Urine normal thereafter. Animal sacrificed 15 days after injection
I. W. 2.98		107 (+1)	5.7 (+1)	0 (+1)			
F. W. 3.10		98 (+4)	3.1 (+4)	23 (+4)			
		64 (+7)	2.1 (+7)	47 (+7)			
		68 (+15)	1.7 (+15)	60 (+15)			

[a] Numbers in parenthesis indicate days in relation to the injection; thus (−2) represents 2 days before injection, (0) on day of injection, (+2) 2 days after injection.
[b] These animals died during the night.
[c] These animals were fed; all others were fasted except for unrestricted water.
[d] The dose is stated as tartaric acid.

Two additional rabbits were studied as controls without drug, as a check against the effect of fasting, as well as of other conditions in the laboratory which might prove unfavorable to the health or renal function of the experimental animals. We soon learned that the slow intravenous injection of fumarate in the doses given was usually without immediate effect on the appearance or the behavior of the rabbits, nor did it produce anorexia. Subsequent experiments were made with rabbits fed 4 ounces of rabbit pellet food and 3 to 4 cabbage leaves daily.

It may be noted that 7 of the 9 rabbits which received a single intravenous dose of the fumarate died. In two cases death resulted from pulmonary edema within 5 and 10 minutes, respectively. In one of these the dose was very large (2 Gm. per Kg. in 15 per cent solution); in the other, 0.5 Gm. in 15 per cent solution per Kg. was injected very rapidly. These effects are probably non-specific and may be due to the sudden change in tonicity of the blood or to direct pulmonary capillary irritation. Of the 5 fasting rabbits in which the injections were made slowly, 2 showed no effects and survived (doses 0.05 and 0.20 Gm. per Kg.) and 3 died after total fasting periods of 5, 6 and 7 days, respectively (doses 0.6, 0.6 and 1.0 Gm. per Kg.). Fasting may have played an important part in these deaths, since the two control rabbits died after fasting 9 and 11 days, respectively. Inasmuch as rabbits Nos. 5 and 10, which were fed throughout and lost practically no weight from the beginning to the end of the experiments, also died in 3 and 11 days, respectively, after the dose, it seems safe to assume that an intravenous injection of disodium fumarate in a dose of 0.5 Gm. or larger is toxic to rabbits and may prove fatal. In those cases in which the mode of death was seen, it frequently involved myoclonic convulsions. At autopsy there were no gross pathological changes to account for the fatal outcome. The mechanism of this form of poisoning remains unexplained. It may also be a non-specific action, since we observed death with clonic convulsions after the intravenous injection of sodium tartrate and of sodium chloride.

Table I shows that after the fumarate there occurred changes in the blood chemistry which might

suggest some renal injury, but the renal effects are, on the whole, equivocal in this group of experiments. Two animals, rabbits 5 and 10, which were fed, showed no significant changes (doses 0.5 and 1.0 Gm. per Kg.). In 2 (rabbits 7 and 8) of the 5 fasting animals there was marked elevation of the N. P. N. The significance of this is problematical, since the 2 control fasting animals (rabbits 1 and 2) also showed a rising N. P. N. and it has been shown by others (4) that fasting itself causes a rise of the blood N. P. N. In rabbit No. 7 with the highest N. P. N. (168 mg.), the blood sample was taken when the animal was nearly moribund, 1 hour and 18 minutes before death.

The histological examination of the kidneys in the case of the animals receiving fumarates showed tubular changes in some cases. These were, however, similar to the changes found in the control fasting rabbit, but in 2 cases (rabbits 8 and 9) they were more marked.

In view of the well-known nephropathic action of the tartrates, the effect of sodium tartrate was studied in 3 rabbits for comparison. A dose of 0.3 Gm. per Kg. caused death with convulsions within 8 hours and no histological changes in the kidneys. A similar dose of 0.3 Gm. to another rabbit caused, within 24 hours, severe tubular degeneration with profound functional changes, as shown by a rise of N. P. N. by about 100 per cent, of the creatinine to about 300 per cent, and a fall of the P. S. P. to 1 per cent. A smaller dose of 0.2 Gm. produced similar but less marked effects. The marked increase in the weight of the kidneys also distinguishes the effects of tartrate from those of the fumarate. The contrast of the histological changes in the kidneys of the tartrate and fumarate animals was very striking.

It is clear from the foregoing experiments that when tested by the single intravenous injection, the nephropathic action of disodium fumarate in rabbits is slight by comparison with tartrate. Whereas a dose of 0.3 Gm. of the tartrate caused severe nephrosis, the effect on the kidney of a dose of 1.0 Gm. of the fumarate was negligible.

Single Dose in Cats.—The above experiments were repeated in cats. The results were essentially similar to those obtained in rabbits. A dose of 0.3 Gm. of

Table II.—The Effect of Repeated Intravenous Doses of Sodium Fumarate and Sodium Chloride

Rabbit No. Initial and final weight (Kg.)	Dose Gm./Kg.	Total No.	Period in Days during Which Drug Was Given	N. P. N.	Creatinine	P. S. P.	Histology Liver	Histology Kidneys	Remarks
16	0.30	7	1–16	43 (1)[a]	1.6 (1)	104 (1)	Marked cloudy swelling	Normal; weight, 14.5 Gm.	Sacrificed on 16th day
I. W. 3.11	NaCl			50 (9)	1.5 (9)	82 (9)			
F. W. 2.73				76 (15)	1.8 (15)	...			
				..	...	90 (16)			
17	0.50	7	1–16	51 (1)	1.9 (1)	105 (1)	Some cloudy swelling	Normal; weight, 13 Gm.	Sacrificed on 16th day
I. W. 2.77	NaCl			45 (9)	1.5 (9)	92 (9)			
F. W. 3.05				46 (15)	1.8 (15)	...			
				..	...	75 (16)			
18	0.20	6	1–11	36 (1)	1.5 (1)	109 (1)	Slight cloudy swelling	Normal; weight, 16.5 Gm.	Sacrificed on 16th day
I. W. 2.60	0.50	3	12–16	45 (9)	1.6 (9)	77 (9)			
F. W. 2.47				49 (15)	1.7 (15)	...			
				..	...	68 (16)			
19	0.30	7	1–16	54 (1)	1.7 (1)	72 (1)	Slight cloudy swelling	Normal; weight, 16.5 Gm.	Sacrificed on 16th day
I. W. 2.74				52 (9)	1.0 (9)	78 (9)			
F. W. 3.09				48 (15)	1.4 (15)	...			
				..	...	87 (16)			
20	0.50	6	1–13	57 (1)	1.6 (1)	97 (1)	Normal	Normal; weight, 14.5 Gm.	Sacrificed on 13th day. Severe infection of soft tissues at site of dye injection for 4 days prior to last blood and P. S. P. tests
I. W. 2.31				38 (9)	1.3 (9)	78 (9)			
F. W. 2.40				37 (13)	1.1 (13)	19 (13)			
3	0.05	5	1–10	38 (3)	1.5 (3)	92 (3)	Normal	Cloudy swelling; tubular degeneration	Died 20 hrs. after last dose in clonic convulsions
I. W. 1.83				50 (10)	1.4 (10)	...			
F. W. 1.74									
4	0.20	13	1–32	33 (3)	1.9 (3)	74 (3)	Fairly marked cloudy swelling	Slight cloudy swelling; weight, 14.5 Gm.	Sacrificed on 32nd day
I. W. 1.86				27 (5)	1.7 (5)	...			
F. W. 2.50				..	...	84 (8)			
				38 (10)	1.4 (10)	67 (10)			
				45 (17)	1.5 (17)	79 (17)			
				65 (25)	2.1 (25)	81 (25)			
				59 (31)	2.1 (31)	...			
				..	...	94 (32)			

[a] Figure in parenthesis represents the day during the period of injection when the test was made.

tartrate per Kg. in one cat caused marked nephropathy with considerable elevation of the blood N. P. N. and creatinine, and a decline of the P. S. P. excretion from 66 per cent to zero. In addition, albuminuria and glycosuria resulted. At autopsy, large scars were found in the kidneys, although in the intervening 15 days the results of chemical tests of the blood and urine had returned to normal. In contrast to these changes, a dose of 1 Gm. of fumarate per Kg. in another cat produced neither chemical nor anatomical changes in the course of 25 days.

Repeated Doses in Rabbits.—Each of 5 rabbits received a series of intravenous injections of sodium fumarate at intervals of 2 to 3 days for periods of from 10 to 32 days. These animals were fed throughout the experiments. The total number of injections ranged from 5 to 13. The single doses ranged from 0.05 (rabbit 3) to 0.5 (rabbit 20) Gm. per Kg.; the total doses received by the different rabbits, from 0.25 to 3.0 Gm. per Kg. The results are summarized in Table II. One of the animals (rabbit 3) died in clonic convulsions on the tenth day after a total dose of 0.25 Gm. per Kg. All the others were well throughout the experiments and survived, although they had received much larger total doses. There were no significant changes in the blood chemistry, urine or P. S. P. excretion in any of the animals. The low P. S. P. excretion, 19 per cent, on the thirteenth day in rabbit 20, was probably due to impaired absorption of the dye as a result of infection at the site of injection. In rabbit 4, the N. P. N. rose from 33 to 65 mg. Whether this was a specific effect of the fumarate is a matter of doubt, since a similar rise was obtained in rabbit 16 which received repeated injections of sodium chloride. The kidneys showed no histological changes, with the exception of those of rabbits 3 and 4 in which there was some cloudy swelling. These animals were carried over from the previous series of experiments in which they had been fasted, and, as has already been indicated, the fasting control also showed cloudy swelling. The experiments with repeated injections of fumarate in non-fasting animals, therefore, give no evidence of nephropathic action.

OBSERVATIONS IN MAN

The laxative action and the toxic effects of sodium fumarate were studied in a group of 26 patients. There were all confined to bed in the hospital for a wide variety of medical and surgical disorders. The description of this group of cases is summarized in Table III. They all suffered with either acute or chronic constipation. Both sexes and a wide range of ages, from 11 to 67 years, are represented. Many of these patients were accustomed to the use of enemas or laxatives, such as milk of magnesia, Ex-lax, cascara, mineral oil, etc., which were taken habitually at intervals ranging from 1 day to 2 weeks.

When they were in need of a laxative, a dose of fumarate was administered. The dose was given after the patients had been without a bowel movement for periods ranging from 21 to 114 hours—in the majority of cases, for a period of 48 hours or longer. The dose was dissolved in half a glass of water and administered orally at bedtime. Each of 10 patients received one dose. In the remaining 16, the recurrence of constipation afforded the opportunity of using the fumarate from 2 to 6 times. The doses ranged from 5 to 30 Gm. The 26 patients received a total of 65 doses. If the bowels did

Table III.—Description of 26 Patients Used in Present Study

Case No.		Age	Sex	Acute or Chronic	Duration of Constipation	Intervals Between Doses in Habitual Use of Laxatives (Days)	Remarks
1	A. D.	31	F	Chronic	5 yrs.	1–2	.....
2	A. E.	19	M	Acute	3 mo.	7	Only when confined to bed
3	M. A.	62	F	Chronic	Many yrs.	10–14	Especially since confined to bed
4	R. B.	50	F	Acute	25 yrs.	...	Only when confined to bed
5	G. B.	25	F	Chronic	5 yrs.	1	.....
6	S. A.	56	F	Acute	1 wk.	...	Only when confined to bed
7	M. C.	71	M	Chronic	Many yrs.	7	.....
8	R. S.	47	F	Acute	1 wk.	...	Only when confined to bed
9	H. D.	48	F	Chronic	Several yrs.	14	.....
10	S. M.	11	F	Acute	1 wk.	...	Only when confined to bed
11	R. L.	59	F	Chronic	Many yrs.	1–2	.....
12	J. P.	67	M	Chronic	25 yrs.	2–3	.....
13	S. F.	59	F	Chronic	Many yrs.	2–4	.....
14	R. O.	46	F	Chronic	Many yrs.	1	.....
15	A. P.	20	M	Acute	4 wks.	4–7	Only when confined to bed
16	J. S.	60	M	Chronic	5 yrs.	2	.....
17	M. B.	18	F	Chronic	Many yrs.	3	.....
18	G. Ba.	28	F	Acute	1 wk.	...	Only when confined to bed
19	R. Br.	14	F	Acute	1 wk.	...	Post-operative
20	E. A.	43	F	Chronic	10 yrs.	2	.....
21	A. R.	22	F	Chronic	8 yrs.	1	.....
22	P. S.	21	F	Acute	2 mo.	3–4	Only when confined to bed
23	A. S.	47	F	Acute	3 days	...	Only when confined to bed
24	E. S.	31	F	Acute	5 wks.	3	Occasional laxative at home
25	B. F.	31	F	Acute	6 wks.	2–3	Only when in bed and when on iron therapy
26	T. S.	24	F	Acute	2 days	...	.....

not move within 24 hours, the dose was considered ineffective.

With the doses used in this study, a bowel movement was produced by 38 or 59 per cent of the 65 doses, and in 18 of the 26 patients. As is the case with all laxatives, the sensitiveness of different individuals to the laxative action varied considerably, some responding to a dose of 5 Gm., while in others a dose of 30 Gm. proved ineffectual. The response to the action of the fumarate varied in the same individual at different times. For example, in case 24, at one time, a dose of 10 Gm. produced a laxative effect in 18 hours; subsequent doses of 20 and 15 Gm. produced no effect, while at still another time a 25-Gm. dose produced a laxative effect in 2 hours.

The laxative effect occurred in an average of 10 hours after the dose, the range being from 1 to 22 hours. With respect to the relation between the speed of the laxative effect and the size of the dose, the results showed that the rapid effects (from 1 to 6 hours after the dose) are fairly evenly distributed between the larger and the smaller doses. Also, the incidence of laxative effects bore no relation to the size of the dose in the range of doses which were used. For example, the 7 doses of 5 Gm. each produced laxative effects in 5 instances, and the 7 doses of 25 Gm. each similarly produced laxative effects in 5 instances.

The character of the stools varied. In some instances it was hard; in others it was soft; and in still others, watery. The loose watery stool was not confined to the larger doses, although the larger doses seemed to account for a larger number of the loose stools.

Disagreeable effects occurred after 10 of the 65 doses. There were slight cramps in 4 cases, nausea in 4, diarrhea in 1, and vomiting in 1. These effects occurred, for the most part, after doses of 15 Gm. or higher.

The urine was examined in a manner similar to that in the animal experiments, after 45 doses of sodium fumarate, and in the case of 26 of these doses, before administration of the drug. The results gave no indication of renal damage.

The serum N. P. N. was determined in 16 instances before and in 38 instances after the administration of fumarate. There were no changes which could be attributed to renal damage as a result of the administration of the drug. In one patient (case 24) there was a microscopic hæmaturia and an N. P. N. of 57 before administration, and values for the N. P. N. of 58 and 47 after administration. In one patient (case 18) the N. P. N. before the drug was 25 Mg. After a dose of 5 Gm., the N. P. N. remained at 25 mg., but after a subsequent dose of 15 Gm., the N. P. N. rose to 39 mg. This rise was in all probability not the result of fumarate poisoning, since this patient had congestive heart failure which was showing signs of progression before and during the experiment.

DISCUSSION

The literature on the toxicity of fumaric acid and its salts is meager. Hermann, *et al.* (1938) (5), found that an approximately 0.7 per cent solution of free fumaric acid injected slowly, intravenously, proved fatal in a dose of 0.47 Gm. per Kg. in rabbits. Death was preceded by a marked fall in the plasma pH, and it is possible that the death was due to the hydrogen ion rather than to the fumarate ion. This, however, is substantially similar to the doses which proved fatal in our experiments. Krebs, *et al.* (1938) (6), observed no ill effects from the slow intravenous injection of 1.1 Gm. of a 2.3 per cent solution of partially neutralized fumaric acid per Kg. in a rabbit. Orten, *et al.* (1937) (7), observed no toxic effects from the intravenous injection of 0.35 Gm. of a 5 per cent solution of sodium fumarate per Kg daily for 3 days in the dog. Ponsford, *et al.* (1932) (8), found the oral dose much less potent in rats since no toxic effects were observed with doses of approximately 17 Gm. of ammonium fumarate per Kg. Our own experiments show that the intravenous injection of sodium fumarate in rabbits is toxic, and proved fatal in one experiment in a total dose as low as 0.25 Gm. per Kg., although the susceptibility to this compound varies greatly; other rabbits survived much larger doses.

We have no knowledge regarding the absorption of fumaric acid. Our experiments in animals were made, therefore, with the intravenous injection, since this simulates complete absorption and provides the most severe test of systemic toxicity. Little nephropathic action was observed after single doses as high as 0.5 Gm. per Kg. and after total amounts from repeated injections, as high as 3.0 Gm. per Kg. The nature of the histological abnormality left the matter open to doubt as to whether any of the changes in the kidney could be attributed to the specific action of the fumarate. In man, we found that single doses as high as 30 Gm. and repeated doses totaling 90 Gm. (0.6 Gm. per Kg. and 1.8 Gm. per Kg. for a 50-Kg. person) produced no changes in urine or blood suggesting nephropathic action.

By comparison with these observations, the nephropathic action of tartaric acid is very high. In our experiments an intravenous dose of 0.2 and 0.3 Gm. of tartaric acid per Kg. caused marked renal effects in the rabbit and the cat. This is in agreement with the findings of Rose (1924) (4) and of Underhill, *et al.* (1914) (9); 1931 (10). The latter authors reported even smaller doses, as low as 0.05 Gm. per Kg., to be nephropathic in the rabbit.

Precise data concerning the toxicity of tartrates in man do not exist. Post (1914) (11) observed no urinary changes in 9 patients following the administration of single doses of 4 to 24 Gm. Connio (1910) (12) reported 2 cases of fatal poisoning in man with severe nephropathy after doses of 50–60 Gm. of tartaric acid, approximately 1 Gm. per Kg. for an average person.

The average dose of tartrate in a Seidlitz powder taken as a laxative is somewhat less than 10 Gm.,

Table IV.—Laxative Effect of Sodium Fumarate in Man

	Case	Time since Last Bowel Movement (Hrs.)	Dose (Gm.)	Laxative Effect: Yes or No	Laxative Effect: Time	Type of Bowel Movement	Disagreeable Effects	Blood N. P. N. Before	Blood N. P. N. After
1	A. D.	48	15	No	46	Normal	None	..	23 (65)[a]
2	A. E.	48	5	Yes	17	Hard to watery in 6 days	Delayed diarrhea	30	29 (120)
3	M. A.	48	15	No	40	....	Nausea, belching, regurgitation	32	21 (120)
4	R. B.	48	10	No	24	....	None	..	...
5	G. B.	48	10	Yes	13	Hard; moderate amount	None	..	...
6	S. A.	87	10	Yes	9	Hard	None	..	21 (45)
7	M. C.	24	5	Yes	9	Normal	None	28	26 (84)
8	R. S.	79	15	Yes	7	Hard, copious	Slight cramps (2 days later)	24	23 (40)
9	H. D.	46	15	Yes	9	Hard to soft	None	21	22 (16)
10	S. M.	53	10	Yes	19	Normal	None	..	...
11	R. L.	48	15	No	28	....	Vomiting (in 2 hrs.)	27	16 (36)
		48	15	No	45	....	None		
12	J. P.	48	10	Yes	13	Hard to soft in 4 days	None	28	28 (96)
		31	15	Yes	13	Soft	None	..	23 (15)
13	S. F.	61	15	No	48	....	None	25	27 (10)
		37	15	No	24	....	None	..	31 (36)
14	R. O.	54	20	No	49	....	Nausea	..	22 (17)
		34	15	No	24	....	None	..	23 (36)
15	A. P.	24	5	Yes	17	Hard; moderate amount	None	25	23 (96)
		43	15	Yes	5	Soft	None	..	24 (15)
		47	15	Yes	1	Normal	None	..	...
16	J. S.	24	10	Yes	7	Hard; moderate amount	None	38	33 (120)
		41	15	Yes	8	Hard; moderate amount	None	..	...
		43	20	Yes	6	Soft	None	..	...
17	M. B.	72	15	No	48	....	None	..	...
		31	15	No	29	....	None	..	...
		64	25	No	54	....	None	..	...
18	G. Ba.	24	5	Yes	5	Hard to soft (3 days)	None	25	25 (17)
		41	15	Yes	1	Soft; semi-solid	None	..	39 (36)
		52	10	Yes	1½	Soft; semi-solid	None	..	...
19	R. Br.	72	10	No	28	Hard with straining	None	..	18 (16)
		21	15	No	24	....	None	..	19 (40)
		60	25	No	24	....	Slight nausea	..	15 (12)
20	E. A.	48	10	Yes	11	Hard; moderate	None	24	20 (45)
		84	20	No	24	....	Cramps	..	18 (12)
		57	30	No	25	....	Slight nausea	..	...
21	A. R.	48	15	Yes	6	Soft; solid	None	..	21 (40)
		48	15	No	20	....	Slight cramps	..	23 (15)
		68	10[b]	Yes	5	Watery	None	..	...
		35	20	No	46	Normal, copious	None	..	...
22	P. S.	44	10	Yes	1	Watery	Diarrhea	19	19 (16)
		37	5	No	18	....	None	..	20 (12)
		55	5[b]	No	24	....	None	..	...
		29	10	No	24	....	None	..	...
23	A. S.	60	10	No	18	....	None	23	25 (85)
		78	10[b]	Yes	18	Hard to soft (3 days)	None	..	...
		46	15	Yes	15	Hard to soft (3 days)	None	..	...
		45	25	Yes	17	Normal	None	..	...
24	E. S.	90	10	Yes	18	Hard; little	None	57	58 (45)
		40	20	No	24	....	None	..	47 (36)
		33	15	No	24	....	None	..	...
		46	25	Yes	2	Soft	None	..	...
		66	30	No	39	Hard	None	..	...
25	B. F.	87	10	No	27	....	None	..	15 (18)
		114	10[b]	Yes	3	Soft	None	..	17 (36)
		46	15	Yes	22	Very hard	None	..	14 (12)
		28	15	Yes	15	Soft	Slight cramps	..	...
		24	15	Yes	21	Normal	None	..	...
		32	25	Yes	2	Soft	None	..	...
26	T. S.	35	5	Yes	21	Normal	None	22	18 (45)
		42	10	Yes	6	Normal	None	..	18 (60)
		46	10	Yes	5	Hard	None	..	16 (12)
		28	15	Yes	14	Normal	None	..	...
		30	25	Yes	11	Soft to semi-solid	None	..	...
		69	25	Yes	3	Soft	None	..	...

[a] Numbers in parenthesis designate the hours after dose when the test was done.
[b] This dose was given immediately after the time designated for the failure of the previous dose.

although not infrequently a double dose, or about 20 Gm., of tartrate is taken. Underhill, *et al.* (1931) (13), observed that after oral administration, a person may absorb as much as 20 per cent of the dose, which in the above case would be 4 Gm. or 0.08 Gm. per Kg. for a 50-Kg. person. This is close to the dose of tartaric acid which may cause injury in the kidney in rabbits (0.05 Gm. per Kg.). The validity of this comparison depends, of course, on the relative susceptibility of the human and rabbit kidney to injury by the tartrates, but concerning this point there are no data.

The use of tartrates in laxative doses apparently does not result in acute renal injury. In any case, we are not aware of any such reports. Nevertheless, in view of the foregoing relationships, the possibility cannot be excluded that the continued use of doses of tartrates which make available to the kidney amount of the order of 0.08 Gm. per Kg. may result in kidney injury which may not be revealed unless suitable long range experiments are devised for this purpose.

Our experiments in man show that sodium fumarate is an effective laxative, and by comparison with the tartrate possesses negligible nephropathic action.

SUMMARY AND CONCLUSIONS

1. Experiments in 22 animals (rabbits and cats) were carried out to study the toxicity and nephropathic activity of sodium fumarate when administered by the intravenous route. The effects were compared with those of sodium chloride and sodium tartrate.

2. The susceptibility of rabbits to sodium fumarate injected intravenously varies greatly. A single dose of 0.5 Gm. per Kg. or higher may be toxic and fatal. Occasionally a smaller dose may prove fatal. On the other hand, repeated injections of 0.5 Gm. totaling 3.0 Gm. over a period of about two weeks may be without ill effects.

3. The study of the kidneys by means of changes in blood N. P. N., creatinine, P. S. P. excretion, urine and histological examination of post-mortem sections shows that the nephropathic action of sodium fumarate is negligible by comparison with that of tartrate.

4. The laxative effect of sodium fumarate was studied in a group of 26 patients suffering from constipation. Oral doses of sodium fumarate, 5 to 30 Gm., produced satisfactory laxative effects within an average of 10 hours after administration. Examination of the urine and blood N. P. N. failed to show any signs of renal damage in this group of patients.

5. The comparison of tartrate and fumarate as laxatives in relation to the possibility of nephropathic action is discussed.

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The Determination of Quinine and the Assay of Quinine and Strychnine in Mixtures*

By Robert L. Herd†

Considerable difficulty has been experienced by chemists in applying the classical acidimetric titration to the determination of quinine. This may be due to the presence in this alkaloid of two amino nitrogens having widely different ionization constants. The customary procedure consists in the titration of the more basic of these amino nitrogens with the formation of the corresponding mono-acidic salt. The buffering action of this salt precludes the accurate determination of the end-point by means of an indicator.

Conant, Hall and Werner (1, 2, 3, 4, 5) found that solutions of weak bases in glacial acetic acid may be successfully titrated with a strong acid, such as perchloric or hydrobromic, made up in the same solvent. They have presented a number of potentiometric titration curves of various bases titrated with glacial acetic acid solutions of perchloric, hydrobromic, hydrochloric and sulfuric acids. Nadeau and Branchen (6) applied this titration method to the estimation of amino acids. The end-point for these titrations was determined potentiometrically or by the use of crystal violet, α-naphtholbenzein or benzoyl auramine as an indicator. Nadeau and Branchen pointed out that acetic acid has a high thermal expansion and, for this reason, they used weight burettes. Since the thermal expansion of acetic acid is a linear function of the temperature, however, titrations with a volume burette have been used in this study by applying the following formula:

$$N_1 = \frac{N_0}{1 + 0.001\ (T_1 - T_0)}$$

where

T_0 = Temperature at which solution was standardized.
T_1 = Temperature at which titration was made.
N_1 = Normality at T_1.
N_0 = Normality at T_0.

In Fig. 1 are shown the electrometric titration curves of quinine in aqueous medium with sulfuric acid, and in glacial acetic acid with perchloric acid in the same solvent. The application of the latter titration to the determination of quinine and its salts will be described later in this communication.

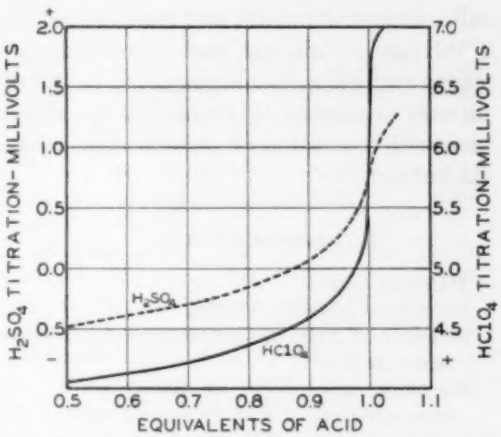

Fig. 1.—Electrometric Titration of Quinine Using a Glass Electrode.
---- Titration with H_2SO_4 in water.
—— Titration with $HClO_4$ in glacial acetic acid.

Quinine and strychnine are frequently encountered together in a wide variety of syrups, elixirs and tablets. The quantitative separation and estimation of these bases have been found to be extremely difficult, owing to the presence of a large quantity of quinine and a relatively small amount of strychnine. Several methods have been proposed for the separation of these alkaloids, all of which give widely divergent results (7, 8, 9, 10). The method most widely used is a modification of the procedure developed by Simonds (11) which was tentatively adopted by the Association of Official Agricultural Chemists (12). Recoveries by titration, as reported by collaborators in this study, varied between 74 and 126 per cent, and 77.4 and 95.9 per cent, respectively, for strychnine and quinine. The low results for quinine were claimed to be due to the difficulty in titrating this base. The gravimetric estimation of quinine, as reported by the

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collaborators, resulted in recoveries ranging between 83 and 95.9 per cent. In addition to the inconsistent results, at least three days are required to complete the assay.

The distribution coefficients of these bases between immiscible solvents did not differ sufficiently to allow their separation by extraction. It seemed possible, however, that products formed by the combination of the bases with an acid might have distribution coefficients which would permit their separation. A study was made of a number of acid salts of these alkaloids, and it was found that a mixture of quinine and strychnine in a strong sulfuric acid solution could be quantitatively separated by extracting the strychnine with a solution of dichloracetic acid in chloroform.

EXPERIMENTAL

Materials Used.—

Acetic acid (glacial)—99.9%.

Indicator—0.2 Gm. α-naphtholbenzein per 100 cc. acetic acid.

Perchloric acid—$N/10$ in glacial acetic. The concentration of acid in reagent quality perchloric acid was determined by titration with sodium hydroxide. A weighed quantity of this acid was placed in a liter volumetric flask, an amount of acetic anhydride to react completely with water present was added and the solution diluted to one liter with acetic acid. This solution was standardized against pure, dry quinine base by the method given below for the estimation of quinine.

Perchloric acid—$N/100$ in glacial acetic. This reagent was prepared by the dilution of the above solution with acetic acid.

Sulfuric acid—$7N$ (approximate).

Dichloracetic acid in chloroform—1%. Chloroform was first washed with water to remove the alcohol.

Determination of Quinine.—A quantity of sample representing approximately 0.5 Gm. of quinine base was accurately weighed and transferred to a separatory funnel containing about 20 cc. of water. An excess of ammonia was added and the alkaloid extracted with five 25-cc. portions of $CHCl_3$. Each extract was filtered through a cotton pledget into a beaker and the combined extracts evaporated to dryness. The residue was dissolved in 20 cc. of acetic acid, 5 drops of indicator added and the solution titrated with $N/10$ perchloric acid to a bluish green end-point. One cc. $N/10$ perchloric acid = 0.0162 Gm. of anhydrous quinine base.

The above method was applied to several purified air-dried salts of quinine. In all cases, the amount of the anhydrous salt in these preparations was established by the determination of the acid radical, and these values were used as the criteria in determining the accuracy of the titration method. The results are given in Table I.

Table I.—Recovery of Quinine from Its Acid Salts

Salt	Quinine Salt Present (*A*) By Determination of Acid Radical	Quinine Salt Present (*B*) By Perchloric Acid Titration	% Recovery $B/A \times 100$
Quinine sulfate	95.73	95.10	
	96.29	95.00	
Average	96.03	95.05	99.0
Quinine hydrochloride	93.79	93.92	
	93.65	93.83	
Average	93.72	93.88	100.2
Quinine dihydrobromide	88.94	89.05	
	88.93	88.98	
Average	88.94	89.02	100.1

Separation of Quinine and Strychnine.—Before these alkaloids could be determined, they had to be separated from interfering ingredients present in the various preparations. To separate the alkaloids from Elixir of Iron, Quinine and Strychnine, N. F., a 200-cc. sample was concentrated on a steam bath to remove alcohol, transferred to a separatory funnel, made alkaline with ammonia and extracted to completion (five times) with 25-cc. portions of chloroform. The combined chloroform extracts were extracted with three 10-cc. portions of sulfuric acid (1–10). The combined acid extracts were made alkaline and reëxtracted with chloroform as above, the chloroform extracts filtered through a pledget of cotton into a 250-cc. beaker and evaporated to dryness. This alkaloidal residue was used for the estimation of quinine and strychnine.

Tablets of quinine, strychnine and reduced iron were ground to pass through a 100-mesh screen, a weighed sample equivalent to 25–50 mg. of strychnine was placed in a separatory funnel fitted with a fritted glass disk (Ace Glass Co., 7290) and macerated for one hour with a mixture of 8 cc. of alcohol and 2 cc. of stronger ammonia T.S. The mixture was then shaken for thirty minutes in a mechanical shaker with 50 cc. of chloroform, the chloroform removed by suction and the residue reëxtracted four times with 50-cc. portions of chloroform, each addition of chloroform followed by a thirty-minute shaking period. The combined chloroform solutions were extracted with three 10-cc. portions of sulfuric acid (1–10), the combined acid solution made alkaline with ammonia and extracted with five 25-cc. portions of chloroform. The chloroform was filtered through a pledget of cotton into a 250-cc. beaker and evaporated to dryness.

The residues thus prepared, or other mixtures of quinine and strychnine free of interfering ingredients, were dissolved in 10 cc. of the dichloracetic-chloroform reagent, transferred to a separatory funnel and the beaker rinsed with small portions of the

Table II.—Separation of Quinine and Strychnine

	Strychnine		Quinine		Ratio of Strychnine to Quinine
	Present, Gm.	Recovered, %	Present, Gm.	Recovered, %	
Mixtures of Quinine and Strychnine	0.0800	97.9	0.8338	100.0	1–10
	0.0800	98.8	1.0528	99.3	1–13
	0.0800	98.0	0.9451	98.7	1–12
	0.0600	97.7	0.8829	99.2	1–15
	0.0600	97.9	1.1243	99.2	1–19
	0.0600	98.0	1.2172	99.2	1–20
	0.0300	96.0	1.3486	99.3	1–45
	0.0300	95.8	1.5823	99.0	1–53
Elixir of Iron Quinine and Strychnine N. F. VI	0.0273	98.1	1.3076	99.7	1–48
	0.0273	97.5	1.3076	99.8	1–48
	0.0273	98.1	1.3076	99.3	1–48
Reduced Iron, Quinine and Strychnine	0.0293	96.3	1.2429	99.6	1–42
	0.0293	97.4	1.2429	99.0	1–42

reagent until the total volume was approximately 25 cc. This solution was shaken for two minutes with 10 cc. of 7*N* sulfuric acid. The chloroform layer was filtered through a pledget of cotton into a second separatory funnel. The acid solution was repeatedly extracted with three 20-cc. portions of the reagent and two 20-cc. portions of washed chloroform. The combined chloroform extracts were shaken through four successive separatory funnels, each containing 5 cc. of 7*N* sulfuric acid. Three 20-cc. portions of the chloroform-dichloracetic acid reagent and two 20-cc. portions of washed chloroform were shaken through the acid solution remaining in these four separatory funnels. The combined chloroform extracts and washings were shaken in a separatory funnel with 20 cc. of ammonia (1–4), the chloroform layer was filtered through a pledget of cotton in a 400-cc. beaker. The ammoniacal solution was washed with a 20-cc. portion of chloroform, the latter filtered and added to the above beaker. The chloroform was evaporated on the steam bath to dryness, care being taken to prevent decrepitation. A 5-cc. portion of acetic acid, to which 5 drops of α-naphtholbenzein indicator had been added, was neutralized with *N*/100 perchloric acid and added to the residue. The resulting solution was titrated with *N*/100 perchloric acid.

The acid solutions and washings from the strychnine separation were combined and carefully neutralized with concentrated ammonia and cooled. The quinine was extracted with five successive 25-cc. portions of chloroform, the extracts filtered through cotton into a 250-cc. beaker. The chloroform was evaporated to dryness, the residue dissolved in acetic acid, transferred to a 100-cc. volumetric flask and diluted to volume. A 50-cc. aliquot of this solution was titrated with *N*/10 perchloric acid using five drops of α-naphtholbenzein solution as an indicator.

1 cc. *N*/100 perchloric acid = 0.00334 Gm. strychnine

This procedure has been applied to various mixtures containing a known amount of quinine and strychnine, and the results are given in Table II.

In the separation of these alkaloids, the sulfuric acid extracts showed considerable fluorescence due to the presence of quinine. The last washing, which ordinarily contained a negligible amount of this alkaloid, usually exhibited a noticeable fluorescence and, with a little experience, this property may be used by the analyst as an indication of the complete extraction of quinine.

As indicated in Fig. 1, the electrometric titration curve of quinine in glacial acetic acid rose quite rapidly at the neutralization point. This property is exhibited by a number of alkaloids and should be of considerable value in their estimation.

SUMMARY

(1) The titration of quinine in glacial acetic acid has been studied and a method for its assay, based on this procedure, has been proposed.

(2) An accurate and convenient method for the separation and determination of quinine and strychnine in various mixtures has been described.

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The Comparative Toxicity and Cathartic Efficiency of Disodium Tartrate and Fumarate, and Magnesium Fumarate, for the Mouse and Rabbit*

By Arthur Locke, R. B. Locke, H. Schlesinger and H. Carr

The availability of fumarate, at this time of serious and increasing shortage in the supply of tartrate, has suggested a comparative survey of the two salts for extent of parallelism in toxicity. Because a considerable use of tartrate is as a saline laxative, the relative cathartic efficiency of fumarate also has been determined.

EXPERIMENTAL

Comparative Toxicity for the Mouse.—Titration was made for the dosage tending to produce one death in ten (LD_{10}), as summarized in Table I.

The salts compared were supplied by Chas. Pfizer and Co. as crystalline preparations with molecular weights of 160 (disodium fumarate), 230 (disodium tartrate) and 228 (magnesium fumarate) and were given in 25% aqueous solution.

The dosages reported are in terms of individual weight. The average weight was between 22 and 23 Gm. The mice were white, of mixed strain and sex, non-fasting and selected only to the extent that use of animals in recognizably poor condition was avoided. A tuberculin syringe fitted into a 16-gage needle, ground sufficiently blunt at the tip to permit harmless insertion into the mouth and throat for a distance of 1 inch, was used for the feedings. Not more than 0.3 cc. was given at one time. Death as a result of feeding accidents was infrequent and distinguishable from death as an effect of the ingested solutions.

The intestines, at death, were distended with fluid. The average weight loss by the survivors was approximately 8 per cent. Ninety per cent of the deaths occurred within 24 hrs.

Toxicity for the Rabbit.—Table II reports a titration for amount of substance producing sublethal impairment in ability to respond to the demands imposed on function by chilling.

The rabbits used were non-fasting New Zealand White males with an average weight between 2.5 and 3.0 Kg. They were not free of the low-grade infections and infestations common to rabbits secured from dealers but had temperatures and weights within the normal range at time of use.

The sodium chloride was Mallinckrodt, C.P. All of the salts were given in 25% aqueous solution, with additional water available.

The warming time is the number of minutes required for a spontaneous recovery of 3 degrees of body temperature by rabbits chilled to 96° F. as described in reference (1). Normally, from 28 to 40 minutes are required in the absence of factors producing change. The warming time tends to shorten during serial chilling (2). It tends to be an average of 6 to 7 minutes shorter following a second chilling before complete recovery from a first and may remain shortened, after two consecutive chillings, for more than 24 hrs. Actions tending to depress or impair functional efficiency cause the warming time to lengthen, instead of shorten, on rechilling (2–4). A lengthening exceeding 40% may be described as "serious" since, in an infected rabbit, it markedly reduces the chance for survival (1–4).

The average dosages producing unmistakable but sublethal effect on function as indicated by the warming time test were 21† (sodium chloride), 19 (disodium fumarate), 16 (disodium tartrate) and 11 (magnesium fumarate) mM/Kg. These levels compare with the following LD_{10} levels found in the mouse: 23 (disodium fumarate), 19 (disodium tartrate) and 10 (magnesium fumarate) mM/Kg.

† See the notations to Tables I and II.

Table I.—The Comparative Mortality Following Single Feedings of Graded Amounts of Disodium Tartrate, Disodium Fumarate and Magnesium Fumarate, to the Mouse

Substance given	Amount, mM/Kg.[a] Range	Av.	Mortality	Approx. LD_{10}, mM/Kg.
Water, only	...	..	0/96	..
Disodium fumarate	10–19	15	2/52	23
	20–29	25	4/32	
	30–34	33	6/24	
	36–48	43	13/29	
	50–66	54	10/14	
Disodium tartrate	7–15	12	2/30	19
	16–23	20	4/35	
	24–43	30	15/51	
Magnesium fumarate	6–12	8	3/36	10
	13–21	15	8/35	
	22–30	28	14/25	
	32–43	36	18/23	
	48–88	63	10/11	

[a] Millimoles per kilogram. For conversion to Gm./Kg., multiply by the molecular weight as given in the adjoining text, and divide by 1000.

* Presented before the Division of Medicinal Chemistry of the American Chemical Society, Atlantic City meeting, 1941. A contribution from the Institute of Pathology of The Western Pennsylvania Hospital, Pittsburgh; aided by a grant from Chas. Pfizer and Co., Inc., New York.

Table II.—The Effects of Single Feedings of Sodium Chloride, Disodium Fumarate and Tartrate and Magnesium Fumarate on the Ability of the Rabbit to Warm up after Chilling

Substance Given	Amount, mM/Kg. Range	Amount, mM/Kg. Av.	Number of Rabbits	Per Cent with a Warming Time >40% Longer 2 Hrs. after	Per Cent with a Warming Time >40% Longer 24 Hrs. after	Per Cent Deaths In <24 Hrs.	Per Cent Deaths 36–48 Hrs.	Per Cent Excreting Fluid with the Feces
None[a]	..	..	17	0	0	0	0	0
Sodium chloride	1–16[b]	7	9	0	0	0	0	0
	17–25	21	7	43	14	0	0	14
	26–45	32	7	86	(0)[c]	43	0	0
Disodium fumarate	2–13	7	8	0	0	0	0	0
	15–23	19	7	29	14	0	0	86
	27–38	33	5	80	(0)[c]	80	0	60
Disodium tartrate	1–12	6	7	0	0	0	0	0
	15–17	16	6	0	17	0	0	0
	20–27	23	7	43	(67)[c]	14	43	43
Magnesium fumarate	9–13	11	22	14	9	0	0	0

[a] Controls. Water and salt were available but not forced.
[b] On a basis of 2 moles of chloride equaling 1 of tartrate, etc.
For conversion of the sodium chloride figures to Gm./Kg., multiply by 0.116, not 0.058.
[c] This figure covers only those of the group not already dead or moribund at the time of the 24-hr. determination.

The effect produced on the warming time by disodium tartrate differed from that produced by the chloride and fumarate in speed of development. Instead of being maximal shortly after ingestion, with rapid subsidence in the absence of fatality, it did not become maximal until the second day. The deaths following single feedings of sodium chloride and fumarate occurred within 24 hrs. Three-fourths of the deaths following tartrate ingestion were during the second day after feeding. Disodium fumarate had a greater tendency to produce fluid excretion with the feces than was observed for disodium tartrate.

The experiment summarized in Table III was undertaken on the presumption that a continuing serious interference with function must culminate in death.

Table III.—Comparative Mortality Following Repeated Feedings of Sodium Chloride and Disodium Fumarate and Tartrate to the Rabbit

Substance	Av. No. of Consecutive Daily Feedings	Amount, mM/Kg.[a] Range	Amount, mM/Kg.[a] Av.	Mortality
Sodium chloride	13	1– 9[b]	5	0/5
	11	17–25	20	0/4
Disodium fumarate	28	2–13	5	0/14
	17, 2[c]	18–23	20	3/6
Disodium tartrate	17	2– 7	5	0/3
	19, 6	15–17	16	3/6

[a] See note b to Table II.
[b] The amount given each rabbit was held constant but was increased from rabbit to rabbit, in each group, over the range indicated.
[c] The figure before the comma refers to the survivors. That following the comma refers to the non-survivors.

The amounts of disodium fumarate and tartrate found to produce a substantial effect on the warming time but no deaths, following a single feeding, produced an incidence of three deaths in six on repeated feeding.

No significantly marked change was detectable in the warming time, weight, blood non-protein nitrogen, creatinine, red cell count or white cell count of the rabbits surviving more than ten repeated feedings.

Seven rabbits sacrificed following 36 consecutive daily feedings, each, of 4 mM/Kg. of disodium fumarate showed no consistently manifested, clearly relevant evidence of injury on gross examination. The results accruing from the gross post-mortem examinations of the fatalities were equally uninterpretable because of the predominance of death within 48 hrs. and during the night, the presence of complicating low-grade infections and infestations and the small total numbers under comparison.

Comparative Cathartic Efficiency for the Mouse.—Titration was made as indicated in Table IV for the dosages producing equivalent shortening of the average time elapsing before the dejection of softened feces, and feces containing charcoal, following feedings of the compared substances together with water and a "test meal" (5).

The mice were not given food after the noon feeding of the day preceding. Fifteen minutes prior to test, 0.15 cc. of water was given in the way described for the toxicity estimations. The test feeding consisted of 0.1 cc. of a mucilage of gum acacia admixed with a thick suspension of charcoal and not more than 0.15 cc. of the salt under test dissolved to a concentration of not more than 25% in water. Following the test feeding, the mice were placed in individual cages floored with white paper. Examination was made of each cage at 15-min. intervals for the presence of soft feces or feces containing charcoal. Water was continuously available. No mouse was used for test more frequently than once in 7–10 days.

The average time elapsing before the first appearance of softened feces was shortened 40% by 1.9 mM/Kg. of magnesium fumarate, 2.7 mM/Kg. of disodium fumarate and 7.4 mM/Kg of disodium tartrate. The average time before first appearance of charcoal was shortened 15% by approximately

Table IV.—Comparative Effectiveness of Magnesium and Sodium Fumarates and of Sodium Tartrate for Producing Softening of the Fecal Contents and Accelerated Dejection in Mice Given Test Feedings of Charcoal Suspended in Water and Gum Acacia

Substance Given	Amount, mM/Kg.	Number of Mice	Av. Time in Hours of First Appearance in the Feces of: Softening	Charcoal
Water,[a] gum acacia alone	..	146	2.84±0.06[b]	3.25±0.04
Magnesium fumarate	3.7	113	1.32±0.04	2.75±0.05
	1.9	56	1.62±0.09	2.93±0.09
	0.9	41	1.81±0.13	2.93±0.12
Disodium fumarate	10.6	32	1.47±0.08	2.36±0.09
	5.3	39	1.36±0.09	2.48±0.09
	2.7	35	1.64±0.14	3.00±0.12
Disodium tartrate	7.4	31	1.65±0.10	2.57±0.09
	3.7	39	2.46±0.16	3.28±0.10

[a] The amount of water given would come to more than a quart if given proportionately to a man.
[b] Probable error as computed from Peter's formula (J. W. Mellor, "Higher Mathematics, etc." (1926), Longmans, Green and Co., New York.)

3.7 mM/Kg. of magnesium fumarate, 4 mM/Kg. of disodium fumarate and 6.5 mM/Kg. of disodium tartrate.

The ratio between the dosage producing a 15% shortening of the "charcoal time" and that producing one death in ten was, for magnesium fumarate and disodium tartrate, near 1/3 and, for disodium fumarate, near 1/4.

Correlative Literature.—Weiss, Downs and Corson (10) report the death of one of two rabbits given 28 and 32 mM/Kg. of sodium fumarate and an additional rabbit given 22 mM/Kg. of sodium tartrate. Death was produced in comparable dosage by sodium citrate, malate, lactate, sulfate and chloride. Sodium potassium tartrate failed to produce grossly apparent injury and little microscopic evidence of injury when fed by Underhill, Wells and Goldschmidt (6) to one fasting rabbit in a dosage of 12 mM/Kg. and to two non-fasting rabbits in dosages of 11 and 13 mM/Kg. Kidney injuries leading to death within 36 hrs. followed feedings of 17–25 mM/Kg. The action on the kidneys was interpreted by Rose (7) to be associated with slow or incomplete oxidation. "Readily oxidizable substances are probably burned to carbon dioxide and water without exerting detrimental renal effects. Acids which are oxidized with difficulty may be disposed of by excretion only. In the course of the body's efforts to eliminate them, damage to the kidney cells may result." Underhill, Leonard, Gross and Jaleski (8) found no indication of oxidation or other alteration of parenterally given tartrate in its passage through the body.

Fumarate is reported to be more readily oxidized than tartrate and actively utilized by tissue as a source of energy (9).

SUMMARY

Disodium fumarate proved to be, on a mole for mole basis, 20% less toxic for the mouse and rabbit than disodium tartrate. Neither substance had a toxicity for the rabbit greatly exceeding that of sodium chloride. Both had an effect on function, measured by the warming time test, which led to fatality on continued feeding in amounts within the range producing the function-impairing effect. Consecutive daily feedings at levels below this range were without significant effect on weight, blood non-protein nitrogen, creatinine, red cell count or white cell count. Disodium tartrate, at levels within the critical range, showed evidence of a delayed toxic effect not observed with the chloride or fumarate. Disodium fumarate produced evidence of cathartic effect on the mouse and rabbit in amounts smaller than were required for equivalent effect by disodium tartrate. Magnesium fumarate, with a cathartic efficiency for the mouse exceeding that of disodium fumarate, had an off-setting, greater toxicity.

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The Quantitative Determination of Methenamine*

By Edmund F. Slowick† and Ray S. Kelley‡

Since the discovery of methenamine, many methods have been suggested for its quantitative determination. These methods depend on one or both of the following properties: (1) methenamine, when hydrolyzed with acids, yields formaldehyde and the ammonium salt of the acid used for the hydrolysis. (2) Methenamine forms addition products with certain chemicals as the halogens, picric acid and uranyl sulfate.

The methods suggested in the literature are slow, tedious and not particularly accurate. In this study an attempt was made to devise a more rapid and accurate method for the assay of methenamine.

The samples used in the work were obtained from reputable manufacturers and were labeled, "Methenamine, U. S. P." In each instance several preliminary assays were performed previous to the determinations reported.

EXPERIMENTAL

The experimental work was divided into two classes: (1) work on certain methods, or on modifications of certain methods, previously reported in the literature; (2) work on methods of assay not previously reported in the literature.

CLASS 1. WORK ON METHODS AND MODIFICATIONS OF METHODS REPORTED IN THE LITERATURE

I. *U. S. P. XI Method.*—About 1 Gm. of methenamine, accurately weighed, is placed in a 400-cc. beaker, 40 cc. of normal sulfuric acid is added and the mixture is heated on a water bath, distilled water being added from time to time as necessary. Heating is continued until the odor of formaldehyde is no longer perceptible. The mixture is cooled, 20 cc. of distilled water is added and the excess acid is titrated with normal sodium hydroxide, using methyl red T.S. as the indicator. Each cc. of normal sulfuric acid is equivalent to 0.03503 Gm. of $(CH_2)_6N_4$.

Table I.—Results Obtained with U. S. P. XI Method

Sample	Wt. of Sample	1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	1.0173	29.15	100.29	550
2	1.0042	28.67	99.92	550
3	1.0059	28.72	99.92	550
4	1.0141	28.98	100.01	550
5	1.0078	28.80	100.01	550

II. *U. S. P. XI Method, Modification A.*—Instead of heating the methenamine and acid on a water bath, direct heat is used with the temperature kept slightly below the boiling point of the mixture. The time required for the assay is thus materially reduced over the time required in the official method of the U. S. P. XI.

Table II.—Results Obtained with U. S. P. XI Method, Modification *A*

Sample	Wt. of Sample	1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	1.0365	29.69	100.37	260
2	1.0209	29.21	100.26	250
3	1.0153	29.09	100.36	250
4	1.0070	28.83	100.29	220
5	1.0138	29.08	100.48	230

III. *U. S. P. XI Method, Modifications B, C, D and E.*—The sample is heated as in Modification *A*, but a change is made in the strength of the sulfuric acid used and the amount of sample is varied to correspond to the strength of the acid. Double-normal, half-normal, fifth-normal and tenth-normal sulfuric acid are used. The results show that decrease in concentration of acid tends to increase the accuracy of the determination and decrease the time required for the assay.

Table III.—Results Obtained with U. S. P. XI Method, Modification *B*

Sample	Wt. of Sample	2*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	2.0228	29.37	101.14	390
2	2.0023	29.43	102.88	380
3	2.0055	29.25	102.00	405
4	2.0164	28.91	100.36	405
5	2.0049	29.02	101.32	380

Table IV.—Results Obtained with U. S. P. XI Method, Modification *C*

Sample	Wt. of Sample	0.5*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.5039	28.81	100.05	210
2	0.5077	29.04	100.09	210
3	0.5071	29.05	100.25	195
4	0.5023	29.18	101.66	180
5	0.5003	28.64	100.17	180

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Table V.—Results Obtained with U. S. P. XI Method, Modification *D*

Sample	Wt. of Sample	0.2*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.2005	28.58	99.77	150
2	0.2005	28.62	99.92	140
3	0.2005	28.52	99.77	135
4	0.2005	28.50	99.50	135
5	0.2005	28.59	99.81	145

Table VI.—Results Obtained with U. S. P. XI Method, Modification *E*

Sample	Wt. of Sample	0.1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.10063	28.60	99.55	120
2	0.10063	28.62	99.62	120
3	0.10063	28.60	99.55	135
4	0.10063	28.65	99.73	120
5	0.10063	28.65	99.73	120

IV. *U. S. P. XI Method, Modification F.*—This Modification differs from the U. S. P. XI Method in that the mixture of sample and acid is placed in a 400-cc. beaker and actively boiled to expel the formaldehyde. This lessens the time required for the assay.

Table VII.—Results Obtained with U. S. P. XI Method, Modification *F*

Sample	Wt. of Sample	1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	1.0290	30.22	100.19	50
2	1.0027	29.47	100.36	50
3	1.0040	29.50	100.34	50
4	1.0041	29.54	100.51	50
5	1.0255	30.10	100.42	55

V. *U. S. P. XI Method, Modification G.*—Tenth-normal sulfuric acid and about 0.1 Gm. of sample are used in this method. In all other respects it is the same as Modification *F*.

Table VIII.—Results Obtained with U. S. P. XI Method, Modification *G*

Sample	Wt. of Sample	0.1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.10049	28.59	99.66	35
2	0.10049	28.59	99.66	35
3	0.10049	28.65	99.87	45
4	0.10049	28.62	99.76	35
5	0.10049	28.60	99.69	30

VI. *Method of Brown and Otten (1).*—About 0.7 Gm. of methenamine is transferred to a Kjeldahl flask and 50 cc. of normal sulfuric acid are added. The flask containing the mixture is connected to a distilling apparatus and, by means of steam, the formaldehyde is distilled into a mixture of 50 cc. of normal potassium hydroxide containing 50 cc. of 3 per cent hydrogen peroxide which is previously rendered neutral to phenolphthalein T.S. After distillation is complete, the receiving flask and contents are heated on a water bath for three hours. The excess alkali is then titrated with normal sulfuric acid, phenolphthalein T.S. being used as the indicator. Each cc. of normal potassium hydroxide is equivalent to 0.0233 Gm. of $(CH_2)_6N_4$. Table IX shows the results so obtained are inconsistent.

Table IX.—Method of Brown and Otten (1)

Sample	Wt. of Sample	1*N* KOH, Cc.	$(CH_2)_6N_4$, %
1	0.7241	30.63	98.56
2	0.6824	28.47	96.91
3	0.7005	28.86	95.99
4	0.7104	31.50	103.03
5	0.7271	33.45	107.17

VII. *Sugiura and Falk (2) Method, Modified.*—Transfer about 0.1 Gm. of methenamine, accurately weighed, to a 100-cc. volumetric flask containing about 10 cc. of distilled water. Add 50 cc. of tenth-normal alcoholic iodine solution and enough distilled water to make 100 cc. Mix, and allow to stand for five minutes. Filter, and titrate 50 cc. of the filtrate with tenth-normal sodium thiosulfate solution, using starch T.S. as the indicator. Each cc. of tenth-normal iodine is equivalent to 0.0035 Gm. of $(CH_2)_6N_4$. Not more than 95.45 per cent of the theoretical amount of methenamine was found in any of the determinations by this method.

Table X.—Method of Sugiura and Falk (2)

Sample	Wt. of Sample	0.1*N* I_2, Cc.	$(CH_2)_6N_4$, %
1	0.0546	12.50	80.19
2	0.0665	14.82	78.00
3	0.0594	16.20	95.45
4	0.0594	15.92	93.63
5	0.0572	14.28	87.37

VIII. *Method of Korostishev'ska (3).*—Dissolve about 1 Gm. of methenamine, accurately weighed, in enough water to make exactly 100 cc. Transfer 5 cc. of this solution to a 150-cc. beaker and, drop by drop with constant agitation, add 30 cc. of tenth-normal iodine solution containing 18 Gm. of potassium iodide per liter. Let stand for five minutes and filter through cotton, discarding the first 5 cc. of the filtrate. Titrate 25 cc. of the subsequent filtrate with tenth-normal sodium thiosulfate, using starch T.S. as the indicator. Each cc. of tenth-normal iodine is equivalent to 0.0035 Gm. of methenamine.

The original work of Korostishev'ska (3) was not available to the writer, and it was, therefore, necessary to follow the directions given in the abstract. It was observed that the first two drops of iodine solution failed to produce a precipitate, and would lead one to suspect that the results obtained would be low due to the slight solubility of the precipitate. However, it will be noted that in the determinations made, the amount of methenamine found exceeds 100 per cent. Thus, the 99.6 to 99.8 per cent accuracy claimed for the method is not realized.

Table XI.—Method of Korostishev'ska (3)

Sample	Wt. of Sample	0.1*N* I_2, Cc.	$(CH_2)_6N_4$, %
1	0.03648	10.92	104.78
2	0.03648	11.09	106.40
3	0.03648	11.06	106.11
4	0.03606	11.07	107.43
5	0.03606	11.01	106.86

IX. *Kjeldahl Determination.*—Accurately weigh about 1 Gm. of methenamine and transfer to a 500-cc. Kjeldahl flask. Add 10 Gm. of powdered potassium sulfate, 0.5 Gm. of powdered cupric sulfate and 20 cc. of sulfuric acid. Digest for thirty minutes, allow the mixture to cool, add 150 cc. of distilled water and then cool again. To the mixture add 150 cc. of 30 per cent aqueous solution of sodium hydroxide in such a way as to cause the solution to flow down the inner side of the flask to form a layer under the acid solution. Add a few pieces of granulated zinc and connect the flask by means of a Kjeldahl connecting bulb with a condenser, the delivery tube from which dips beneath the surface of a mixture of 40 cc. of half-normal sulfuric acid and 25 cc. of distilled water contained in a 400-cc. beaker. Distil and, after distillation is complete, add 1 drop of methyl red T.S. to the contents of the receiving flask and determine the excess acid by titration with half-normal sodium hydroxide. Each cc. of half-normal sulfuric acid is equivalent to 0.007 Gm. of nitrogen or 0.0175 Gm. of $(CH_2)_6N_4$.

Table XII.—Kjeldahl Determination

Sample	Wt. of Sample	0.5*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Total N_2, %
1	0.5149	29.28	99.51	39.85
2	0.5469	31.10	99.51	39.85
3	0.5168	29.32	99.28	39.69
4	0.5304	30.22	99.70	39.88
5	0.5039	28.68	99.60	39.83

CLASS 2. WORK ON METHODS NOT REPORTED IN THE LITERATURE

I. *Decomposition of Methenamine with Acid and Oxidation of the Formaldehyde Formed with a Solution of Hydrogen Peroxide.*—Procedure: Dissolve about 0.1 Gm. of methenamine in 15 cc. of normal sulfuric acid and add 50 cc. of 3 per cent hydrogen peroxide, previously rendered neutral to phenolphthalein T.S. Warm the mixture and allow to stand for ten minutes at about 90° C. Now add 40 cc. of normal sodium hydroxide and gently boil the solution until all of the ammonia is expelled, adding distilled water from time to time to keep the volume constant. Titrate the excess of sodium hydroxide with normal sulfuric acid. Each cc. of normal sodium hydroxide is equivalent to 0.0233 Gm. of $(CH_2)_6N_4$.

As will be noted from the determinations reported in Table XIII, the results of this assay were consistently low and, after numerous attempts, the method was abandoned.

Table XIII.—Oxidation Method with Hydrogen Peroxide

Sample	Wt. of Sample	1*N* NaOH, Cc.	$(CH_2)_6N_4$, %
1	0.1193	3.2	62.50
2	0.1155	4.4	88.80
3	0.1140	4.4	89.90
4	0.1095	3.9	82.90
5	0.1364	5.9	101.07

II. *Argentometric Method.*—Transfer from 0.1 to 0.2 Gm. of methenamine to a 250-cc. Erlenmeyer flask containing about 20 cc. of distilled water. Add about 1 Gm. of potassium chlorate, 50 cc. of tenth-normal silver nitrate and 2 cc. of nitric acid T.S. Heat the mixture on a water bath for thirty minutes. Filter the solution and wash the precipitate with distilled water until free of silver nitrate. Titrate the combined filtrate and washings with tenth-normal ammonium thiocyanate, using ferric ammonium sulfate as the indicator. Each cc. of tenth-normal silver nitrate is equivalent to 0.007 Gm. of $(CH_2)_6N_4$. Results of this assay, as indicated in Table XIV, were not satisfactory and the method was discontinued.

Table XIV.—Argentometric Method

Sample	Wt. of Sample	0.1*N* $AgNO_3$, Cc.	$(CH_2)_6N_4$, %
1	0.2232	38.31	123.28
2	0.2286	42.10	128.91
3	0.1136	21.92	135.07
4	0.1075	20.53	133.68
5	0.1010	18.33	127.03

III. *Potassium Bromate Method.*—Spitzer (4) determined formaldehyde using a solution of potassium bromate. An attempt was made to apply the same method to the formaldehyde formed by the hydrolysis of methenamine. The results were so unsatisfactory that this method was discarded.

IV. *Alkaline Hypobromite Method.*—Ammonium compounds, treated with sodium hypobromite, liberate nitrogen. Methenamine, digested with sulfuric acid, decomposes and ammonium sulfate is one of the products of decomposition. A combination of these two reactions seemed to point to a method for the assay of methenamine.

Preparation of sodium hypobromite solution: Dissolve 125 Gm. of sodium bromide in 200 cc. of distilled water in a 1000-cc. volumetric flask, add 125 Gm. of bromine and enough distilled water to make 1000 cc. Mix thoroughly. Add 10 cc. of the bromine solution to 10 cc. of sodium hydroxide, containing 25 Gm. of U. S. P. sodium hydroxide in each 100 cc., and dilute the mixture to 100 cc. with distilled water.

Assay: Gently boil 1 Gm. of methenamine with 25 cc. of sulfuric acid T.S. contained in a 250-cc. beaker until the odor of formaldehyde is no longer perceptible. Cool, transfer to a 200-cc. volumetric flask, add distilled water to the mark and mix thoroughly. Pipette 10 cc. of this dilution into a 250-cc. glass-stoppered bottle containing 25 cc. of the sodium hypobromide solution and allow to stand for fifteen minutes, shaking intermittently. Now add 3 Gm. of potassium iodide and an excess of acetic acid (about 15 cc.). Titrate the liberated iodine with tenth-normal sodium thiosulfate, using starch T.S. as the indicator. At the same time carry out a blank test using exactly the same quantities of reagents. The difference in the number of cc. of tenth-normal sodium thiosulfate consumed in the

actual test and in the blank test, multiplied by 0.1167 and divided by the weight of sample taken, gives the per cent of $(CH_2)_6N_4$ in the sample.

Table XV shows that the results obtained by the Alkaline Hypobromite Method exceeded 100 per cent of methenamine. This may be explained by stating that the iodine color consistently returned after an apparent end-point was reached in the titration. In an attempt to overcome this difficulty, the titration was carried on in an atmosphere of carbon dioxide but the same difficulty was still encountered.

Table XV.—Alkaline Hypobromite Method

Sample	Wt. of Sample	0.1*N* $NaBrO_3$, Cc.	$(CH_2)_6N_4$, %
1	0.05025	43.29	100.53
2	0.05025	43.47	100.95
3	0.05025	43.15	100.20
4	0.05025	43.26	100.46
5	0.05025	43.15	100.20

V. *Calcium Hypochlorite Method.*—In the presence of sodium bicarbonate and bromide, ammonia is oxidized quantitatively to nitrogen and water by calcium hypochlorite (5).

$$2NH_3 + 3OBr^- = N_2 + 3Br + 3H_2O$$

After the conversion of methenamine to ammonium sulfate, a method of assay was tried which was based on the reaction between the ammonium sulfate and calcium hypochlorite. The results were accurate and the assay could be performed in about thirty minutes.

Preparation of calcium hypochlorite solution: Mix about 10 Gm. of calcium hypochlorite with about 200 cc. of water and allow to stand for five minutes. Filter the solution into a flask and add enough distilled water to the filtrate to make about 1000 cc. The solution prepared should be at least tenth-normal when titrated with sodium thiosulfate as directed in the assay.

Assay: Accurately weigh from 0.95 to 1.05 Gm. of sample, transfer it to a 200-cc. volumetric flask and add distilled water to the mark. When the sample has dissolved, pipette exactly 10 cc. of the dilution into a glass-stoppered flask, and add 10 cc. of distilled water and 10 cc. of sulfuric acid T.S. Boil the solution vigorously, adding a little distilled water from time to time, until the odor of formaldehyde is no longer perceptible. Cool, add one drop of methyl red T.S., neutralize with potassium bicarbonate, 1 : 10 and then add 3 Gm. of potassium bromide. While gently agitating the flask, slowly add 45 cc. of calcium hypochlorite solution. Stopper the flask and let stand for five minutes with occasional shaking. Remove the stopper and add 3 Gm. of potassium iodide, previously dissolved in 15 cc. of 36 per cent acetic acid. Titrate the liberated iodine with tenth-normal sodium thiosulfate, using starch T.S. as the indicator. Run a blank, omitting the sulfuric acid and potassium bicarbonate but using exactly the same quantities of the other reagents. The difference in the number of cc. of tenth-normal sodium thiosulfate consumed in the actual test and in the blank, multiplied by 0.1167 and divided by the weight of sample used, gives the per cent of $(CH_2)_6N_4$ in the sample.

Notes on the assay: The time necessary for the hydrolysis of the sample is fifteen to twenty minutes. Potassium bicarbonate is added until the solution is neutral to methyl red indicator because, if strongly acid, free bromine will be liberated and, if too alkaline, the end-point is indefinite and high results are obtained.

Table XVI.—Calcium Hypochlorite Method

Sample	Wt. of Sample	0.1*N* $Ca(OCl)_2$, Cc.	$(CH_2)_6N_4$, %
1	0.05025	42.98	99.81
2	0.05025	42.88	99.57
3	0.05025	43.03	99.92
4	0.05025	42.98	99.81
5	0.05025	42.98	99.81
6	0.05025	43.04	99.95
7	0.05025	43.07	100.01
8	0.05025	42.92	99.78
9	0.05025	43.01	99.88
10	0.05025	43.03	99.92

VI. *Tablets Containing 5 gr. of Methenamine and 5 gr. of Sodium Acid Phosphate Were Assayed by the Calcium Hypochlorite Method.*—About 1 Gm. of the powder, representing about 0.5 Gm. of methenamine, was accurately weighed and dissolved in enough water to make 200 cc. A portion of this dilution was treated in the same manner as in the previous (V) method. Since there is only about half as much of methenamine, a total of 20 cc. was used. Table XVII shows the accuracy of this method.

Table XVII.—Assay of Tablets

Sample	Wt. of 10 Tabs.	Wt. of Sample	0.1*N* $Ca(OCl)_2$, Cc.	Gm. of $(CH_2)_6N_4$ per Tablet
1	6.1888	0.10022	45.31	0.3265
2	6.1888	0.10022	45.15	0.3253
3	6.1888	0.10022	45.31	0.3265
4	6.1888	0.10022	45.41	0.3271
5	6.1888	0.10022	45.40	0.3270

CONCLUSIONS

1. Assay of methenamine by the U. S. P. XI method is slow and unsatisfactory. Greater accuracy may be obtained by using less sample and tenth-normal solutions.

2. The results obtained by the precipitation methods that were tried were inaccurate.

3. The use of hydrogen peroxide and potassium bromate as oxidizing agents gave poor results.

4. The alkaline hypobromite method was rapid but, possibly due to excess alkalinity, the method was not entirely satisfactory.

5. Of the methods tried, the calcium hypochlorite method seems best suited for the assay of methenamine and certain mixtures containing methenamine. The method is simple, comparatively rapid and accurate.

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Electrophoretic Separation of the Blood Pressure Principles of Hog Kidney Extracts*

By Raymond Jonnard and Marvin R. Thompson

It has been shown by various authors that the ischemic kidney of various animals yields, under suitable conditions, a vaso-pressor substance called "renin" (1, 2, 3, 4, 5). This substance is also present in the normal organ (4, 6) and is somewhat specific (8, 10). It is neutralized by a vaso-depressor substance extractable under certain conditions (7, 9). The work done to date indicates that the chemical properties of these substances have some similarity, so that their complete separation is difficult. Thus they are usually obtained together in solution during the preliminary stages of the extraction of the kidneys.

Much stress has been laid upon the biological properties of these two antagonistic principles, but the literature contains only very limited information on the physical and chemical properties of the substances involved. Most of the alleged properties of these substances have been inferred from solubility data and from the conditions of their extraction. Although it can be safely assumed, to date, that "renin" is a protein-like substance, little can be said as yet of the nature of the depressor principle.

In view of the findings by du Vigneaud and co-workers (11) that the vaso-depressor factor extracted from the hypophysis gland could be isolated in the catholyte compartment of an electrophoretic apparatus, and that this substance seems to have an iso-electric point far in the alkaline range, it was thought desirable to submit certain kidney extracts to a similar electrophoretic analysis.

In view of the uncertainty in the identification of the various kidney depressor principles claimed by various authors (6, 12, 13, 14), we have used several different types of kidney extracts.

EXPERIMENTAL

METHOD

1. Electrophoresis.—Due to the minute amount of active material contained in the hog kidney, this active material is usually obtained in the form of dilute solutions as will be discussed later. Therefore, a rather large electrophoretic cell had to be designed in order to separate the components of a volume of solution large enough to yield material for repeated bioassays. The apparatus was made of large flanged Pyrex glass fittings (15); it was somewhat similar to that of Russell and Stauffer (16). The clamps holding the various parts were also used to squeeze removable diaphragms of either paper or cellophane. Both anodic and cathodic compartments had a capacity of 90 cc. The medium cell held 200 cc. The electrode compartments held 300 cc. The anodic and cathodic compartments were separated from the central cell by paper diaphragms and from the electrode compartments by cellophane diaphragms preventing the escape of the practically non-dialyzable vaso-motor principles in the present series of experiments. The whole apparatus was held on a "flexaframe" and could be immersed in a bath of running water at any given temperature. The assembly is shown in Fig. 1.

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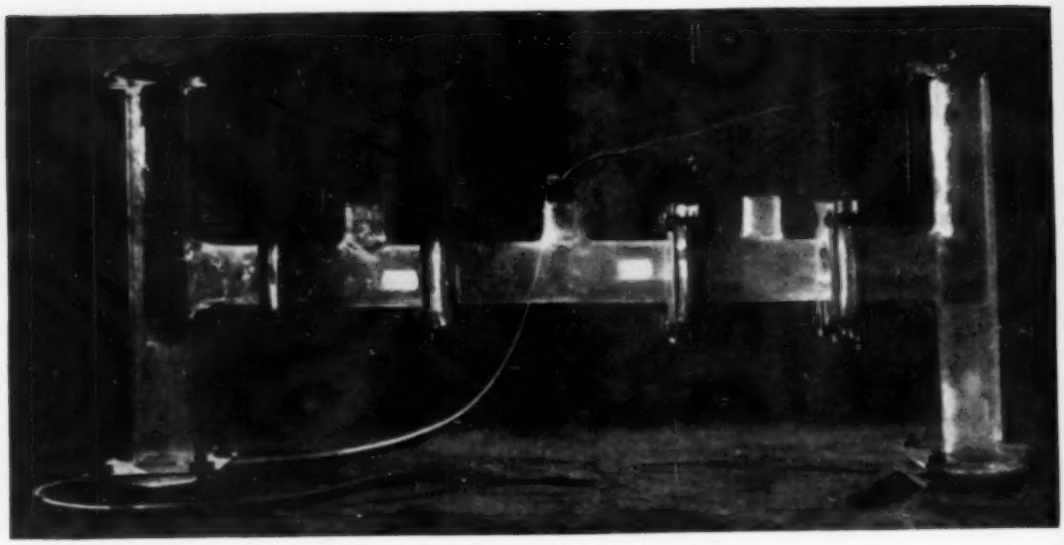

Fig. 1.—The Electrophoretic Apparatus.

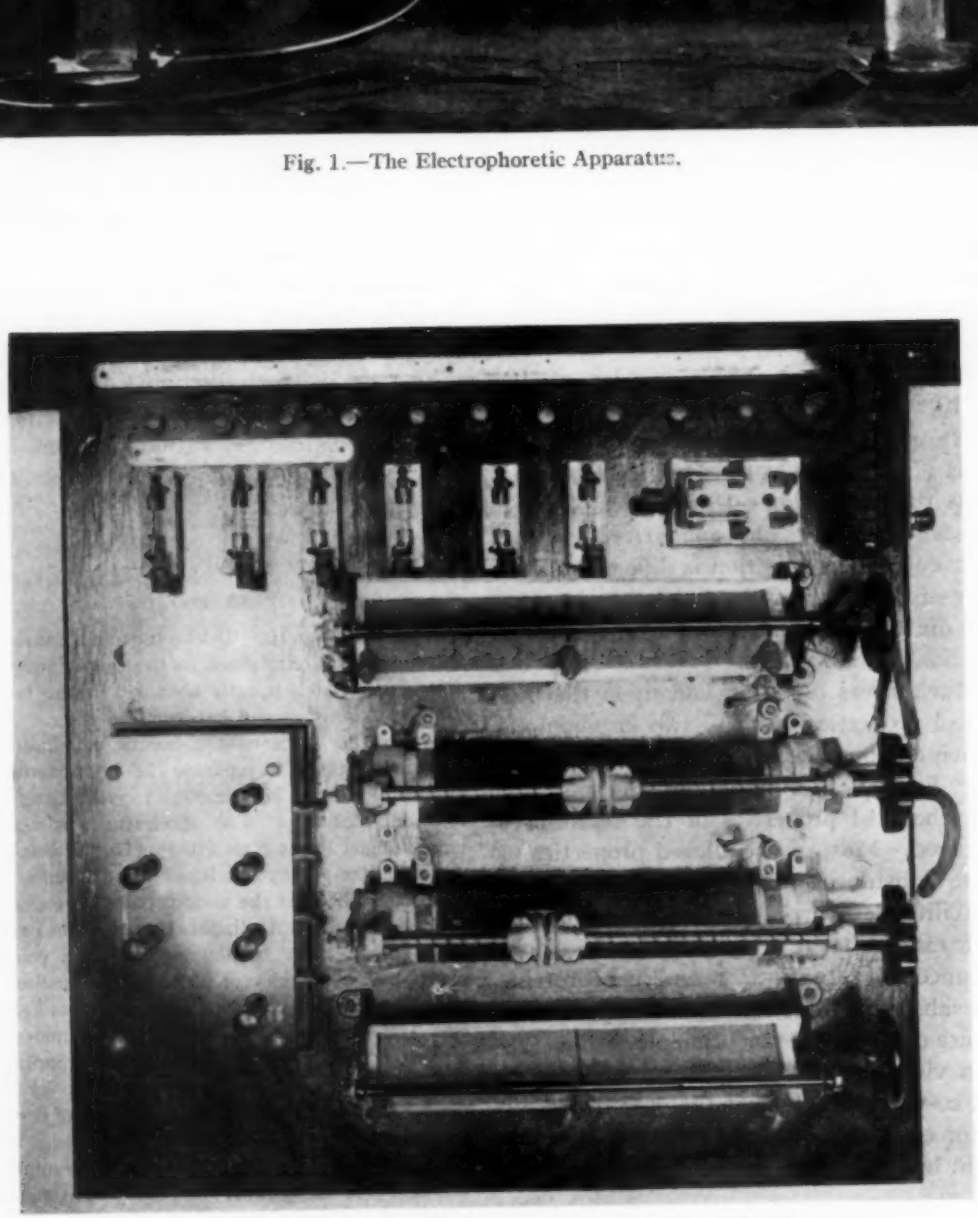

Fig. 2.—Electrical Control Panel for the Electrophoretic Cell.

The electrical circuit included a voltmeter and an ammeter used for preliminary adjustment, and a control board comprising a twin sliding nichrome wire potentiometer in parallel with a twin carbon resistor-potentiometer, thus providing a very great sensitivity in the adjustment of both potential and intensity together with an almost complete independence of the resistor values from the effect of external thermic and hygrometric factors. The current source was a constant speed d.-c. generator. The control panel is shown in Fig. 2. Accurate measurements of both potential and intensity were also made with a Leeds and Northrup type K potentiometer set-up.

The potential gradient p in our apparatus is given by the formula used by Tiselius (17): $p = (i/q.k)$ (volt/cm.), where i = intensity in amp.; q = cross-section area of the electrophoretic tube in cm.^{2} (here = 7.5 cm.^{2}); k = conductivity of the solutions in ohms -1. Notwithstanding the inherent sources of inaccuracy in our set-up particularly designed more for separating large volumes of material than for determining boundary positions or velocities of migration, no attempt has been made at determining the k's separately. Rather the k values have been approximately obtained from a direct measure of both the potential v and the intensity i by the relation $r = v/i$ and $k = (l/r \times C^{te}$, where $C^{te} = r/0.1118$ if N KCl is used for the calibration of the apparatus. The values of p thus found are given in the accompanying table. This method is somewhat similar to that of Briggs (18).

With such a form of electrophoretic apparatus, it might be possible that a poor heat transfer would bring about disturbances due to the thermal gradient inside the tubes. It is known that in such a type of apparatus the thermal gradient, $dt/dr = wr/2k$, considerably increases with the radius r since $t_0 - t = w.r^2/4k$ (r = radius of the tube, here 12 mm.; k = thermal conductivity of the liquids; w = load, in watt/cc.). Here again the effect of the thermal gradient upon the velocity of the migrating molecules and the completeness of the separation could be neglected, but, nevertheless, this gradient was kept low by using a very low load, w (see table), to avoid a thermal alteration of the active principles, which have been shown to be thermolabile (Page (6), Braun-Menendez (10)). Such precaution was important in this experiment when the joule effect $w^{watt} = ri^2t$ could have been important due to the large value of both r and t (see table for values of t).

Finally it should be pointed out that in our instrument, the relationship indicated by Tiselius between the volume of the electrode compartment and the volume of solution to be separated (17), was not satisfied with the result that the ionic composition of the liquids in the various compartments of the apparatus underwent slight progressive change during the experiments. It has been verified, however, that the pH remained constant within 0.1 unit, while the load = w^{watt} could be continuously adjusted. Since in most cases the active principles were further isolated, sometimes in the dry form, as will be discussed later, this source of error was neglected during this preliminary investigation.

II. Preparation of the Solutions.—(*a*) The saline extracts were prepared at 0° C. using 1 liter of saline per Kg. of fresh kidney. One cc. represented 1 Gm. of fresh kidney.

(*b*) The defatted kidney extracts were prepared according to the method of Page and others: extraction of the minced kidneys with four parts of acetone per weight, and with one part of ether. The prepared kidney powder thus obtained was finally extracted with one part of saline solution at 0° C. which was made up to 1 mol. in sodium phosphate and filtered: 1 cc. represented 0.5 Gm. of fresh kidney.

The "renin D" was prepared according to Page's method (8). Doses representing 5 Gm. of fresh kidney were uniformly used for the bioassay.

The "renin E" was Page's "renin D" further purified by dialysis at pH = 4, neutralization, precipitation by $^1/_2$ saturation with ammonium sulfate at pH = 3.5, re-precipitation by saturation with sodium chloride at pH = 2.0, and drying. Doses of 0.1 mg. per Kg. of body weight were uniformly used for the bioassay.

All the solutions were finally made up to 1 mol. in sodium phosphate and adjusted at various pH values recorded in the table. Finally they were dialyzed 16 hours in cellophane bags against the same buffer solution at the chosen pH value just before the electrophoresis.

III. Isolation of the Separated Material.—In some cases the anolyte and catholyte were directly tested. When the isolation of the active material was attempted, the liquid from both compartments was brought to pH = 2.0 with lactic acid and saturated with ammonium sulfate at 0° C. It is known that under such conditions the "renin" precipitates, while vaso-dilator substances of the type isolated by Grollman (19), for instance, are also rendered insoluble.

IV. Identification of the Isolated Products.—The most practical criterion so far available for the identification of the substances involved in this study is the effect upon the blood pressure of anesthesized animals. The tests were conducted upon either normal cats or normal dogs under pentobarbital anesthesia. The blood pressure was recorded from the carotid artery in the conventional manner, the solutions under test being injected intravenously. In many instances rapid control experiments have been made by inserting a needle into the femoral artery of the animal, the needle being connected directly to a direct reading Bourdon or Thycos manometer, the test solutions being injected into the jugular vein. Although the latter method does not leave any record of the experiment, it has been found rapid, convenient and reliable, and gives results identical to those obtained with the former method.

Figure 3 shows a few kymographic records which have been considered typical of the substances under investigation: (*a*) a typical increase of blood pres-

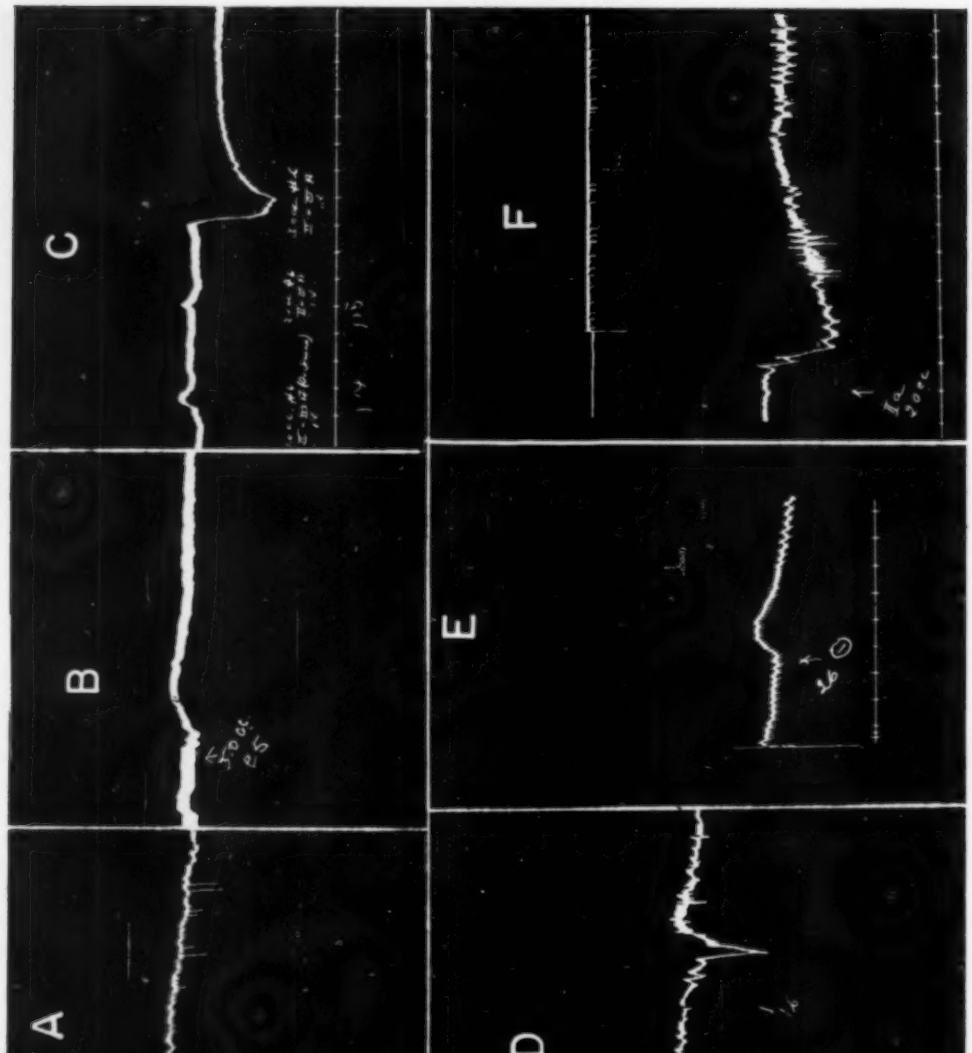

Fig. 3.—Blood Pressure Tracings (see Text).

sure obtained with "renin E" (0.2 mg./Kg.), (*b*) a similar curve obtained with "renin D," (*c*) a typical decrease of blood pressure obtained with a kidney depressor fraction obtained by Grollmann's method, (*d*) a non-typical decrease of blood pressure frequently obtained with depressor or toxic substances of various origin (such results must not be confused with the typical response to the injection of "renin" or of the specific kidney hypotension factor), (*e*) an increase of blood pressure obtained with a cathodic electrophoretic fraction at pH = 6.5, (*f*) a decrease of blood pressure obtained with an anodic electrophoretic fraction at pH = 5.0. The records were similar for cats and dogs.

RESULTS

The series of measurements was necessarily limited because of (*a*) the length of time required for preparing potent kidney extracts in any reasonable quantity, and the fact that such extracts are rather unstable, (*b*) the time required for the many biological tests which must of necessity be done without delay after electrophoretic separation or isolation of the separated active materials.

Table I.—Effect of the Electrophoretic Fractions upon the Blood Pressure of Anesthesized Dogs (Expts. I to V) and Cats (Expts. VI to IX)

Preparations Tested	Experiment No.	Temperature, °C.	t, Hours	v, Volts	i, Amp.	k	p	pH	Blood Pressure Anolyte	Blood Pressure Catholyte
One mol. sodium phosphate solution of kidney extract; final precipitation with 30% sodium chloride at a pH of 2.0 and redispersion in saline	I	20	24	10	0.1	0.062	0.21	6.5	0	+45
	II	20	48	10	0.1	0.062	0.21	6.5	+30	−35
	III	20	36	10	0.1	0.062	0.21	6.5	−30	+15
	IV	15	72	10	0.1	0.062	0.21	6.5	−30	+10
Same, with addition of chloroform	V	15	72	10	0.1	0.062	0.21	6.5	−20	+25
Saline extract of non-defatted kidneys	VI	15	48	5	0.05	0.062	0.11	7.5	0	−15
						0.062	0.11	6.5	−40	+45
						0.062	0.11	5.0	−20	+45
						0.062	0.11	3.0	0	0
Saline extract of defatted kidneys	VII	15	48	5	0.05	0.062	0.11	7.5	−20	0
						0.062	0.11	6.5	−50	+50
						0.062	0.11	5.0	−20	+60
						0.062	0.11	3.0	0	0
Renin D	VIII	15	36	7.5	0.1	0.083	0.15	6.5	−25	+30
Renin E	IX	15	36	7.5	0.1	0.083	0.15	6.5	0	+35
						0.083	0.15	5.0	−10	+55
						0.083	0.15	3.0	0	0

The results are shown in the accompanying table. The sign (−) indicates a decrease of blood pressure, and conversely; the amplitude of the blood pressure variation observed is expressed in terms of mm. of Hg.

DISCUSSION

The conditions of the experiments would eventually permit the evaluation of the mobilities $u+$ and $u-$ of the active substances investigated. Indeed, the velocity of migration in cm./sec. is given by: $i.u/q.k = pu$, where p is the potential gradient defined above. The total distance traveled by each of the charged particles after a time t sec. is given by: $d(\text{cm.}) = p.u.t$, so that after the time: t sec., each particle has swept through a volume $V = p.u.t.q = u.i.t./k$. Unfortunately, in the absence of a basis for a quantitative evaluation of the active components from either chemical data or the bio tests, such calculation cannot be exactly related to the findings reported in the accompanying table. Nevertheless, the above-reported results lead to some interesting conclusions.

The essential results of this investigation show the possibility of a sharp separation of the antagonistic vaso-motor principles contained in the kidney extracts, by means of an electrophoretic field. Quite consistently the pressor factor has been found in the catholyte compartment (from which it could be further isolated in the dry form) when the migration took place at pH values ranging between 3.0 and 7.0. It has also been shown that "renin D" and "renin E" behave similarly. At a pH below 3.0 the pressor factor can no longer be detected under the conditions of the experiment.

On the other hand, the anolyte has likewise been found quite consistently to contain a substance producing a decrease of blood pressure, when the electrophoresis was conducted at pH values ranging between 5.0 and 7.5. From a comparison of Experiments I, V, VI and VII it seems that this depressor substance has an isoelectric point not far from neutrality. A comparison of Experiments I, III and VIII makes it appear that the pressor principle, and probably the "renin," migrates much more rapidly than the depressor substance at pH values ranging between 5.0 and 6.5.

The similarity of the blood pressure curves obtained with the "renin D" and "renin E" and the catholyte on one hand, and with the Grollman depressor substance and the anolyte on the other hand, is very striking and suggests a likely chemical relationship.

If the above results are confirmed, it would appear that the vaso-motor substances extractable from the kidney are fundamentally different from those of the hypophysis (11) or the brain (20).

The work is being pursued with the object of determining the exact isoelectric points of the substances considered and the conditions of their quantitative isolation.

SUMMARY AND CONCLUSIONS

1. The electrophoretic field has been used for the separation of the kidney vaso-pressor from the vaso-depressor principles.

2. The vaso-pressor substance migrates to the cathode at pH values ranging between 3.0 and 6.5 under the conditions of the experiment. It has been found that "renin D" and "renin E" have a similar behavior under the same conditions.

3. The vaso-depressor factor consistently migrates to the anode at pH values

ranging between 5.0 and 7.5, and its migration velocity is much slower than that of the pressor factors.

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The Determination of Theobromine in Tablet Mixtures*

By A. G. Richardson and Y. C. Campbell

In an effort to find a suitable rapid method for the determination of the alkaloid theobromine in tablet mixtures containing other nitrogenous organic compounds it became plainly evident that the ordinary shake-out methods using two immiscible solvents were unsatisfactory. This was confirmed by E. C. Deal (1) who recommended the Emery-Spencer (2) method wherein the theobromine in a tablet mixture is precipitated with iodine; phenobarbital or other barbiturates being uneffected. He pointed out, however, that starch, which is present in most tablet mixtures, would interfere and produce inaccurate results.

The A. O. A. C. volumetric method (3), in which the silver salt of theobromine is precipitated and the nitric acid formed from a known amount of standard silver nitrate

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thereby is titrated, does not proceed satisfactorily in the presence of other nitrogenous compounds which form silver salts easily.

Taking advantage of the alkaloidal precipitant phosphotungstic acid, preliminary test-tube experiments revealed that a copious yellow-orange precipitate could be obtained with this reagent. Roughly, quantitative experiments showed that the precipitate did not form with phenobarbital.

This led to the method used in this work. The method used is, briefly, the gravimetric precipitation of the insoluble compound formed by the action of phosphatododecatungstic acid (4) on hot strongly acidified (5) solutions of the tablet mixtures containing theobromine or its salts. The precipitate is filtered while hot, washed, dried and weighed.

The reaction for this precipitation is probably as follows:

$$P_2O_5.24WO_3 + 7H_2O + 6C_7H_8O_2N_4 \longrightarrow 2H_7P(W_2O_7)_6.(C_7H_8O_2N_4)_3$$

The precipitate obtained from a typical assay was analyzed to determine its composition. *Analysis.*—Calcd. for $C_{21}H_{31}O_{43}N_{12}W_{12}P$: N, 4.86; W, 63.83. Found: N, 4.85, 4.84, W, 63.48, 63.37. These data and the findings of Heiduschka and Wolf (6), who have found that one mol of the acid combines with 3 mols of most alkaloids, indicate that the precipitate is $P(W_2O_7)_6H_7.(C_7H_8O_2N_4)_3$.

EXPERIMENTAL

Reagent.—Phosphatododecatungstic acid or simple phosphotungstic acid is prepared by dissolving 103.6 Gm. of sodium tungstate $Na_2WO_4.2H_2O$ with 13.75 Gm. 85% H_3PO_4 and a sufficient volume of distilled water to make 500 cc. of reagent.

Technique.—Twenty or more tablets were reduced to a fine powder and dried to constant weight. A quantity of the dried material equivalent to approximately 0.2 Gm. of theobromine alkaloid was accurately weighed and transferred to a 250-cc. beaker. To this 100 cc. of distilled water were added followed by 5 cc. of concentrated H_2SO_4. This mixture was heated to boiling to dissolve the theobromine. (The usual tablet mixtures will dissolve completely in this hot acid solution with perhaps a slight opalescence. If at this stage any insoluble matter is present, filter the hot solution through a Gooch crucible and wash the residue thoroughly with boiling water uniting the filtrate and washings.) To the boiling hot solution was added reagent phosphotungstic acid drop by drop until precipitation was complete, then 10 per cent excess of reagent was added (usually a total of approximately 8 cc.). The mixture thus produced was digested four hours on the steam bath, then filtered while hot through a tared Gooch crucible having a thick asbestos mat. The precipitate was transferred quantitatively to the Gooch crucible and washed with a total of not more than 15 cc. of approximately $N/10$ H_2SO_4 followed by 4 1-cc. portions of cold distilled water, dried to constant weight at 110° C., cooled and weighed. The weight of the dried precipitate multiplied by 0.15627 equaled the weight of theobromine in the original sample.

The following table shows the results obtained when the various tablet mixtures were assayed for theobromine using the above technique. A total of 25 Gm. of each mixture was prepared by weighing the U. S. P. or C.P. ingredients accurately on the analytical balance. Each mixture was triturated thoroughly.

Table I.—Results of Assays for Theobromine on Known Mixtures

Tablet Mixtures		Assays for Theobromine
No. 1		
Theobromine salicylate	68.74%	100.23
Calcium salicylate	11.46%	100.17
Phenobarbital	2.86%	99.67
Lactose	9.55%	99.83
Starch	6.44%	100.30
Calcium stearate	0.96%	
No. 2		
Theobromine salicylate	42.96%	100.50
Calcium salicylate	11.46%	99.52
Phenobarbital	28.64%	100.42
Lactose	10.02%	100.71
Starch	6.44%	
Calcium stearate	0.48%	
No. 3		
Theobromine alkaloid	41.44%	96.79
Potassium iodide	41.44%	95.75
Phenobarbital	4.13%	99.74
Starch	4.94%	99.76
Lactose	7.31%	105.09
Calcium stearate	0.74%	102.50
No. 4		
Theobromine alkaloid	41.44%	100.74
Acetophenetidin	41.44%	100.45
Phenobarbital	4.13%	101.13
Starch	4.94%	100.05
Lactose	7.31%	
Calcium stearate	0.74%	
No. 5		
Theobromine with sodium salicylate	83.68%	100.52
Phenobarbital	2.86%	100.92
Starch	6.44%	100.70
Lactose	6.54%	100.10
Calcium stearate	0.48%	100.60
No. 6		
Theobromine salicylate	68.74%	
Calcium salicylate	11.46%	
Barbital	2.86%	100.41
Lactose	10.02%	100.61
Starch	6.44%	
Calcium stearate	0.48%	

The table given below shows the results of the assays made on tablets that can be purchased on the open market. These assays, of course, show quite a variation on account of the variations of manufacturing technique.

Table II.—Results of Assays for Theobromine on Manufactured Tablets Purchasable on the Open Market

Tablet Mixtures		Assays for Theobromine
No. 1		
Theobromine salicylate	68.74%	99.71
Calcium salicylate	11.46%	99.69
Phenobarbital	2.86%	99.20
Tablet excipients	16.94%	99.85
		98.62
		99.13
No. 2		
Theobromine with calcium salicylate	85.20%	99.55
Phenobarbital	2.84%	100.49
Tablet excipients	11.96%	100.83
No. 3		
Theobromine	69.66%	100.44
Phenobarbital	3.48%	99.83
Tablet excipients	26.86%	100.42
		99.78

Discussion of Results.—It is obvious that phenobarbital and other nitrogenous, non-alkaloidal substances have practically no effect upon this gravimetric precipitation. Iodides cause a marked interference. The method is applicable to mixtures containing theobromine alkaloid, theobromine salicylate, theobromine with sodium salicylate and theobromine calcium salicylate.

It was noticed that mixtures containing the free alkaloid had a tendency to form finer precipitates. The effect of this tendency may be overcome somewhat by longer digestion and by the use of sintered glass crucibles in place of Gooch crucibles with asbestos mats.

The precipitate on the filter in one determination was dried and weighed, then washed with cold $N/10$, H_2SO_4 a few cubic centimeters at a time until 100 cc. had been passed through, then re-weighed after drying to constant weight again. By this procedure, which resembles the usual washing technique, it was found that approximately 5 mg. of the precipitate on the filter were dissolved. The precipitates usually weigh approximately 1.2400 Gm. Therefore, the loss in weight from careful washing is quite low.

It is to be remembered that phosphatododecatungstic acid is an alkaloidal precipitant, and that if other alkaloids are present in the tablet mixtures they will be precipitated along with the theobromine.

SUMMARY

1. A method is proposed for the assay of theobromine and its salts in tablet mixtures.

2. The method is rapid and reasonably accurate.

3. The assay is satisfactory even though theobromine may be present with other nitrogenous substances such as the barbiturates.

4. The common tablet diluents, excipients and lubricants do not interfere with the assays.

5. Tablet mixtures containing iodides cannot be assayed satisfactorily by this method.

REFERENCES

(1) Deal, E. C., 56th A. O. A. C. Convention Paper No. 113 (1940).

(2) Emery, W. O., and Spencer, G. C., *Ind. Eng. Chem.*, 10 (1918), 605.

(3) "A. O. A. C. Methods of Analysis" (1940), page 593.

(4) Mellor, J. W., "A Comprehensive Treatise of Inorganic Theoretical Chemistry," 11 (1931), page 862.

(5) "Allen's Commercial Organic Analysis," 6 (1912), page 593.

(6) Heiduschka, A., and Wolf, L., *Schweiz. Apoth.-Ztg.*, 58 (1920), 213–218, 229–233.

Book Reviews

Food Analysis, by A. G. WOODMAN, Associate Professor of Analytical Chemistry, Emeritus, Massachusetts Institute of Technology. 4th Edition. 607 pages. 1941. McGraw-Hill Book Company, Inc. Price $4.00

The scope of the book remains the same as that of the third edition. The new edition has been enlarged by 50 pages. The first three chapters are devoted to the consideration of general methods; the microscopical examination of foods; and food colors and preservatives. The remaining eight chapters consider the methods of analysis and the interpretation of the analytical results obtained for milk, cream and ice cream; edible oils and fats; carbohydrate foods; cocoa and chocolate; spices; cider vinegar; flavoring extracts; and alcoholic foods.

Since no new chapters were added the slight enlargement is distributed throughout the contents. Fourteen pages were added to the chapter on alcoholic foods, the section on whiskey undergoing the major change. Other additions include a discussion of photometers; a brief section on ice cream; a re-

vision of the methods for sugar determinations on cocoa and chocolate; a method for the determination of volatile oil in spices by direct measurement; and a method for the determination of the oxidation value of vinegars to aid in distinguishing between distilled vinegar and "artificial vinegar." The section on "permitted dyes" has been brought up to date and the names adopted in the regulations of the Food, Drug, and Cosmetic Act have been added. Less important methods have been deleted.

The author has again given the references to many of the methods of analysis suggested, and also a list of selected references at the close of each chapter.

The book is written "chiefly for students and beginners" and is well adapted for instructional and laboratory use. Its use as a reference also adds value to this book.—CHARLES W. BLIVEN

The Chemistry of Organic Medicinal Products, by GLENN L. JENKINS, Dean and Professor of Pharmaceutical Chemistry, Purdue University, and WALTER H. HARTUNG, Professor of Pharmaceutical Chemistry, University of Maryland. xii + 457 pp., 78 figs., 16.5 x 24 cm. 1941. John S. Swift Co., Inc., 2100 Locust St., St. Louis, Missouri. Price, $3.80.

There has been a need for an introductory volume that presents a systematic classification and a survey of the compounds in common use as medicinals. Therefore, this volume will prove most interesting and valuable to anyone interested in medicinal chemistry.

The text is divided into fifteen chapters, fourteen of which present the most important of the synthetic and natural drug products according to accepted chemical classification. The last chapter deals with stereoisomerism including asymmetric synthesis and physiological activity of the optical isomerides. The bibliography throughout is quite representative and good for a volume of such broad scope. A number of useful tables of miscellaneous character (physical properties, chemical classes of compounds, etc.) are included. The volume in general is readable and quite ably written. Although the text is written for students having basic training in chemistry, a good deal of space is devoted to nomenclature, preparation and general properties of each of the chemical classes of compounds discussed. Perhaps some of this space might have been used to better advantage for developing some of the more important topics (phosphoric acid esters, sulfonamide drugs, proteins, etc.) that are treated briefly.

As may be expected in a first edition, there is a comparatively large number of errors. However, considering the number of structural formulas and the general nature of the contents, the percentage of errors is not excessive. Several of the chemical formulas are incorrect, for example: santonin, page 210; acetarsone, page 326; pyridium, page 257; scarlet red dyes, page 256; the tetranucleotide, page 194; and cholesterol, page 447. The structures for certain other substances are written in a manner no longer accepted, for example: the acridine dyes, pages 373–375; ring structure for betaines, pages 358, 398; quaternary nitrogen compounds and nitro compounds are shown with 5 covalent bonds for nitrogen. In addition, quite a number of typographical errors appear, which likely accounts for the erroneous chemical names assigned to: cacodylic acid, page 322; arsphenamine, pages 327–328; scarlet red sulfonate, page 256, etc.

A number of compounds of biological and pharmacological importance do not lend themselves well to a rigid chemical classification, *e. g.*, cozymase, thiamine, azochloramide, etc. A classification on a basis of general pharmacologic properties and use would appear to offer a distinct advantage in such cases.

It is hoped that in subsequent editions a more complete index will be provided. Fluorescein, for example, appears in the index only under the name of soluble fluorescein; synthalin, although mentioned under guanidine derivatives, and nylon, which is also mentioned in the text, do not appear in the index.—T. C. DANIELS

Materia Medica and Pharmacology, an Introductory Text, by CLAYTON S. SMITH, Ph.D., M.D., and HELEN L. WIKOFF, M.S., Ph.D. College Book Company, Columbus, Ohio, 1941.

This text is of the most elementary nature, written principally for classes in nursing. It employs mimeograph style printing, contains no tables, charts or illustrations, and lacks running page chapter headings.

The Materia Medica and Pharmacology are considered simultaneously, an approach which does not permit coördinated presentation of either one. The introduction contains brief discussions of definitions, official pharmaceutical preparations, dosage, administration of drugs and prescription writing. Part I consists of drugs acting before absorption, Part II drugs acting after absorption and Part III contains a discussion of arithmetic, percentage solutions and calculation of dosage. An appendix discusses sulfonamide drugs.

Parts I and II are organized by chapter headings with no further classifications of the drugs according to their pharmacological action. Symptoms of drug action, rather than basic pharmacological action, is the principal topic. The text is essentially accurate, but its superficial nature, with omission of any consideration of the fate of drugs, the autonomic nervous system as a unit, or the theory of chemical mediation, and the brevity of discussions on general principles of pharmacology such as synergism, antagonism and types of drug action, impose a tremendous limitation upon its usefulness. While it may be satisfactory for its intended use, it would be of little value for classes with even a minimum scientific background.—LLOYD W. HAZLETON

Pharmacology and Dental Therapeutics, by HERMANN PRINZ, A.M., D.D.S., M.D., Sc.D., Dr. Med. Dent., and U. GARFIELD RICKERT, A.M., D.D.S. Eighth edition, revised by Edward C. Dobbs, D.D.S. The C. V. Mosby Company, St. Louis, Mo., 1941. 507 pp. 44 figs. 15 x 23 cm. Price, $6.50.

To those acquainted with this text the change of title from "Dental Materia Medica and Therapeutics" to the present one will be welcomed. In line with this change of title the reviser has materially changed the organization from three parts to fifteen chapters.

The first portion of the book deals with the history of pharmacology and therapeutics which is followed by a consideration of classification, compounding and administration of drugs, and posology.

The material on locally acting drugs is adequate and should be of value to students, practitioners and pharmacists interested in supplying dental preparations and who desire information on the special uses of drugs in dentistry.

Aside from the chapter on general anesthetics the material on drugs which act after absorption is rather inadequate from the pharmacological point of view, although the dental therapeutic application is good and includes uses, doses and concentrations of the various agents.

Some of the pharmacological aspects which may be questioned are the classification used, inclusion of alcohol as a cerebral stimulant of importance, paralysis or diminished activity of the vasomotor centers principally accomplished by the administration of the nitrites, discussion of "adrenergic" drugs without explanation of the term or correlation with cholinergic drugs.

The book may be well recommended as a guide to dental therapy and dental preparations but, as is often the case when two major subjects are included in one text, the material is insufficient for the study of the fundamental principles of pharmacology.—L. W. HAZLETON

Essentials of Endocrinology, by ARTHUR GROLLMAN, Ph.D., M.D. J. B. Lippincott Company, Philadelphia, Pa., 1941. xvi + 480 pp. 74 figs. 15 x 23 cm. Price, $6.00.

In this excellent book the author presents a well-documented survey of the field of endocrinology. The organization is such that the reader with some background in biological science will find it both useful and enjoyable.

The preface of the book embraces a basic survey of the scope, history and methods of endocrinology, the nature and method of administering hormones, etc.

The text is divided into five parts: (1) endocrine glands of the cranial cavity; (2) branchiogenic organs; (3) endocrine organs of the abdominal cavity; (4) hormones of the reproductive organs; (5) hormones derived from non-endocrine organs. Under the individual gland or hormone are discussions, where applicable, on orientation, anatomy, histology, pathology, disease, chemistry, assay, pharmacology and clinical use. Throughout the book the evaluation of these subjects is based on both experimental and clinical observations, which the author justly considers inseparable.

Of additional interest to pharmacists and practitioners is the inclusion, in the front and back lining of the book, of a table giving the substance, scientific name, commercial designation, standardization and units, and text reference page of the commercially available endocrine products.

The comprehensive scope of the book and inclusion of the most important references make it a *must* in the library of the professional pharmacist and as a reference for students.—L. W. HAZLETON

Books Received

To January 1, 1942

Accepted Dental Remedies. Seventh edition. Council on Dental Therapeutics, American Dental Association, 212 East Superior Street, Chicago, Ill., 1941. 309 pp. $1.00.

EMIL J. BELANGER, *Drug and Specialty Formulas*. Chemical Publishing Company, Inc., Brooklyn, N. Y., 1941. 307 pp. $6.00.

H. BENNETT, Editor-in-Chief, *The Chemical Formulary*. Vol. V. Chemical Publishing Company, Inc., Brooklyn, N. Y., 1941. 676 pp. $6.00.

WILLIAM BOYD, *An Introduction to Medical Science*. Second edition. Lea and Febiger, Washington Square, Philadelphia, Pa., 1941. 358 pp. $3.50.

A. J. CLARK, *Applied Pharmacology*. Seventh edition. The Blakiston Company, Philadelphia Pa., 1941. 672 pp. $5.50.

J. HOWARD CRUM, *Beauty and Health, a Course in Loveliness*. Smith and Durrell, New York, N. Y., 1941. 336 pp. $2.00.

MALCOLM DOLE, *The Glass Electrode*. John Wiley and Sons, Inc., New York, N. Y., 1941. 332 pp. $4.50.

WALTER H. EDDY and GILBERT DALLDORF, *The Avitaminoses*. Second edition. The Williams and Wilkins Company, Baltimore, Md., 1941. 519 pp. $4.50.

ARTHUR L. FAUBEL, *Cork and the American Cork Industry*. Revised edition. Cork Institute of America, 25 West Forty-third Street, New York, N. Y., 1941. 151 pp. $1.50.

REUBEN FRIEDMAN, *Scabies—Civil and Military*. Froben Press, New York, N. Y., 1941. 288 pp. $3.00.

ARTHUR GROLLMAN, *Essentials of Endocrinology*. J. B. Lippincott Company, Philadelphia, Pa., 1941. 480 pp. $6.00.

SEALE HARRIS and SEALE HARRIS, JR., *Clinical Pellagra*. The C. V. Mosby Company, St. Louis, Mo., 1941. 494 pp. $7.00.

JOHN W. HASSLER, *Active Carbon, the Modern Purifier*. Copyright, 1941, by Industrial Chemical Sales Division, West Virginia Pulp and Paper Company. 157 pp.

HARRY N. HOLMES, *General Chemistry*. Fourth edition. The Macmillan Company, New York, N. Y., 1941. 720 pp. $3.75.

R. G. HOSKINS, *Endocrinology, the Glands and Their Functions*. W. W. Norton and Company, Inc., 70 Fifth Avenue, New York, N. Y., 1941. 388 pp. $4.00.

ERNEST HAMLIN HUNTRESS and SAMUEL PARSONS MULLIKEN, *Identification of Pure Organic Compounds*. John Wiley and Sons, Inc., New York, N. Y., 1941. 691 pp. $7.50.

GLENN L. JENKINS and WALTER H. HARTUNG, *The Chemistry of Organic Medicinal Products*. The John S. Swift Company, Inc., 2100 Locust Street, St. Louis, Mo., 1941. 457 pp. $3.80.

EDWIN O. JORDAN and WILLIAM BURROWS, *Textbook of Bacteriology*. Thirteenth edition, revised. W. B. Saunders Company, West Washington Square, Philadelphia, Pa., 1941. 731 pp. $6.00.

ELLIOTT P. JOSLIN, *A Diabetic Manual*. Seventh edition. Lea and Febiger, Philadelphia, Pa., 1941. 238 pp. $2.00.

I. M. KOLTHOFF and H. A. LAITINEN, *pH and Electro Titrations*. Second edition. John Wiley and Sons, Inc., New York, N. Y., 1941. 190 pp. $3.00.

I. M. KOLTHOFF and J. J. LINGANE, *Polarography*. Interscience Publishers, Inc., 215 Fourth Avenue, New York, N. Y., 1941. 510 pp. $6.00.

ELDIN V. LYNN, *Organic Chemistry with Applications to Pharmacy and Medicine*. Lea and Febiger, Philadelphia, Pa., 1941. 410 pp. $4.50.

WILLIAM H. O. MCGEHEE and MELVIN W. GREEN, *A Textbook of Dental Pharmacology, Materia Dentica and Pharmaco-Therapeutics*. Second edition. The Blakiston Company, Philadelphia, Pa., 1941. 462 pp. $5.00.

(*To be continued*)

U. S. Pharmacopoeial Convention

Statement of Income and Expense for the First Year of the 1940–1950 Decennial Period from April 15, 1940, to April 30, 1941*,†

The accounts of the U. S. Pharmacopœial Convention, covering the period from April 15, 1940, to April 30, 1941, have been audited by R. G. Rankin and Company, Certified Public Accountants, New York and Washington. By direction of the U. S. P. Board of Trustees, copies of the Statement of Income and Expense and of the Statement of Cash Receipts and Disbursements as prepared by the auditors are attached together with supplemental tables giving further details with respect to disbursements.

The returns for the year from the sales of the U. S. P. XI in English and Spanish have been larger than was expected. The returns from the sales of reference standards continue to increase, and indicate that these reference standards serve a very useful purpose.

The expenses of this period include, in addition to the cost of the Decennial Meeting of the Convention, and a meeting of the retiring Executive Committee, the cost of a meeting of the General Committee of Revision, a special meeting of the entire Sub-Committee on Scope, and three meetings of the Board of Trustees. A meeting of the special committee to revise the Constitution and By-Laws as authorized by the Convention and a meeting of a sub-committee on drafting have likewise been held during the year in order to forward the completion of the work.

The plan to issue the U. S. P. XII as promptly as possible in conformity with the new five-year program approved by the Convention of 1940 is also reflected in the expenses of this first year of revision. An Executive Assistant to the Chairman of the Committee of Revision has been provided; the special meeting of the Sub-Committee on Scope was held in order to complete the list of admissions and deletions; special meetings of the Hormone Advisory Board, the Sterile Products Advisory Board, and

* Copies of this statement may be obtained by writing to Secretary Adley B. Nichols, 43rd St. and Kingsessing Avenue, Philadelphia, Pa.

† Published at the request of the Board of Trustees of the United States Pharmacopœial Convention as a matter of record.

the Blood and Blood Substitutes Advisory Board, the members of which serve without compensation excepting for expenses, were required to complete the special assignments given to them; full-time technical assistants were continued for the Chairmen of the Sub-Committees on Organic and on Inorganic Chemicals, which Sub-Committees are responsible for about 62 per cent of all U. S. P. XII tests; and the clerical assistance, office supplies and postage have increased, since about twice as much circular and bulletin material, including certain papers read at the Pre-Convention Meeting, has been issued as compared to the first year of revision in the preceding decade.

While the program for the earlier issuance of the U. S. P. XII under the five-year plan has brought many expenses of revision, previously spread over a longer period, into the first year, it is also expected to insure an earlier return of supporting funds.

For the U. S. P. Convention,
W. PAUL BRIGGS, *Treasurer*
For the U. S. P. Board of Trustees,
E. F. KELLY, *Chairman*
ADLEY B. NICHOLS, *Secretary*

U. S. PHARMACOPŒIAL CONVENTION, WASHINGTON, D. C.

STATEMENT OF INCOME AND EXPENSE FOR THE PERIOD FROM APRIL 15, 1940, TO APRIL 30, 1941

Income				
Sale of Pharmacopœias				
Collections			$20,989.82	
Less: Decrease in Accounts Receivable				
April 15, 1940		$2,743.92		
April 30, 1941		2,349.31	394.61	$20,595.21
Sale of Reference Standards, A. M. A. Articles, etc.				
Collections			$ 4,718.62	
Collections Pending Remittance to the Treasurer			288.75	
			$ 5,007.37	
Less: Decrease in Accounts Receivable				
April 15, 1940		$ 376.58		
April 30, 1941		270.46	106.12	4,901.25
Interest on Investments				
Collections			$ 1,510.15	
Adjustment of Accrued Interest			62.61	
			$ 1,572.76	
Less: Amortization of Bond Premiums			273.02	1,299.74
Use of U. S. Pharmacopœia Text by Others				40.00
Miscellaneous Income				363.25
Total Income				$27,199.45
Less:				
Expense				
Cost of Publications Sold		$4,114.83		
Publication and Sales Expense				
Add: Decrease in Inventory				
April 15, 1940	$12,635.45			
April 30, 1941	5,056.86	7,578.59	$11,693.42	
Administration			3,580.53	
Convention			2,293.35	
Revision			29,458.85	
Research			6,152.42	
Expenditures from Petty Cash Fund Pending Reimbursement from the General Account			833.28	
			$54,011.85	
Less				
Furnishings and Equipment Capitalized (at 75% of cost)		$ 233.36		
Stationery and Supplies Inventory Adjustment		66.83	300.19	53,711.66
Excess of Expense over Income				
For the Period from April 15, 1940, to April 30, 1941—Exhibit "A"				$26,512.21

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS (GENERAL ACCOUNT) FOR THE PERIOD FROM APRIL 15, 1940, TO APRIL 30, 1941

Cash on Deposit, April 15, 1940		$15,770.31
Add:		
Receipts		
Sale of Pharmacopœias	$20,989.82	
Sale of Reference Standards, A. M. A. Articles, etc.	4,718.62	
Interest on Investments	1,510.15	
Use of U. S. Pharmacopœia Text by Others	40.00	
Miscellaneous Income	363.25	
Loans:		
Bank	10,000.00	
Petty Cash Fund	550.00	38,171.84
		$53,942.15

Less:

Disbursements

	Publication and Sales	Administration	Convention	Revision	Research	Total
Printing	$ 948.70	...	$ 332.64	...	...	$ 1,281.34
Binding	2,591.38	...	...	...	...	2,591.38
General	574.75	315.98	286.29	3,655.12	34.26	4,866.40
Clerical	...	889.60	803.89	7,626.48	157.85	9,477.82
Meetings	...	902.20	...	7,970.75	1,107.92	9,980.87
Supplies	...	199.11	172.84	2,505.29	1,022.91	3,900.15
Post. & Tel.	...	136.14	57.81	1,151.21	33.75	1,378.91
Honoraria and Salaries	...	1,137.50	...	6,550.00	...	7,687.50
Exhibits	...	...	639.88	...	...	639.88
Compensation	...	...	...	...	3,470.37	3,470.37
Materials	...	...	...	...	325.36	325.36
Total	$4,114.83	$3,580.53	$2,293.35	$29,458.85	$6,152.42	$45,599.98

Repayment of Loan from Petty Cash Fund	550.00	46,149.98
Cash on Deposit, April 30, 1941—Exhibit "A"		$ 7,792.17

A MORE DETAILED AND SUPPLEMENTARY FINANCIAL STATEMENT OF THE REVISION EXPENSES

REVISION—MEETINGS

MEETINGS OF THE GENERAL AND EXECUTIVE COMMITTEES OF REVISION, AND OF SUB-COMMITTEES, SPECIAL CONFERENCE GROUPS, ETC.

General Committee Conference (1940)	$4,334.32
Sub-Committee on Scope Conference (1941)	1,611.26
Retiring Executive Committee Meeting (May 1940)	703.10
Traveling, hotel, and incidental expenses of the General Revision Chairman, for conferences with Sub-Committee Chairmen, Sub-Committee groups, Food and Drug Officials, and others assisting in the Revision	601.53
Pre-Convention Meeting, May, 1940	596.29
Advisory Boards, including Clerical Help	124.25
Total	$7,970.75

REVISION—CLERICAL

Stenographic and clerical assistance, Chairman's office	$7,626.48

REVISION—SUPPLIES

Stationery for the General Chairman and for all members of the Committee of Revision	$ 265.71
Binders for all official circulars and correspondence	503.93
Mimeograph supplies (paper for circulars, stencils, ink and maintenance)	887.41
Secretarial and other revision supplies	592.56
Typewriters	255.68
Total	$2,505.29

REVISION—POST. AND TEL.

Postage, Express, Telephone and Telegraph Service, Chairman's office	$1,151.21

REVISION—GENERAL EXPENSES

Translating the "Pharmacopœia and Physician" Series into Spanish	$ 266.95
Spanish Abstract of Second U. S. P. XI Supplement	158.19
Printing and Binding 1011 copies of First Series of "Pharmacopœia and Physician" articles in Spanish	833.68
Contribution to the A. PH. A. toward an Abstract of Pharmacopœial literature	1,000.00
U. S. P. Exhibit at the A. M. A. Convention, 1940	66.25
Extending U. S. P. information by exhibits (other than A. M. A.) by publishing 2000 sets of A. M. A. reprints of the Second "Pharmacopœia and Physician" Series	841.90
Dues for Membership in National Council on Pharmaceutical Research	25.00
Dues for Inter-Society Color Conference	25.00
Miscellaneous expenses	438.15
Total	$3,655.12

REVISION—HONORARIA AND SALARIES

Revision Chairman, E. Fullerton Cook	$4,175.00
Executive Assistant to Chairman, Adley B. Nichols	2,100.00
Walter A. Bastedo (As acting Chairman of Sub-Committee on Scope 1937–1940)	250.00
Honoraria to author of article in "Pharmacopœia and Physician" Series (for clerical and stenographic assistance)	25.00
Total	$6,550.00

RESEARCH

All expenses of Sub-Committee and Advisory Board activity, incurred in the routine of preparing texts for U. S. P. XII, are charged as Research.

Sub-Committee No.	Meetings, Supplies, Postage, Clerical, and General	Technical Assistance	
3—Biological Assays	$ 5.00		
5—Botany and Pharmacognosy	15.60		
6—Proximate Assays	68.16	Mr. E. E. Hartman[a]	$ 250.00
7—Inorganic Chemicals	281.14	Dr. F. K. Bell[b]	1,558.37
8—Organic Chemicals	813.68	Mrs. F. F. Johnson and Miss Elinor Sackter[c]	1,500.00
9—Reagents, Test Solutions, and Tables	23.60	Mr. Harold Urist[d]	87.00
10—Volatile Oils	5.00		
Hormone Advisory Board	420.65		
Sterile Products Advisory Board	232.42		
Anti-anemia Advisory Board	28.50		
Blood and Blood Substitutes Advisory Board	64.50		
Digitalis Preservation Study	59.35	Dr. Donald P. LeGalley[e]	40.00
Posterior Pituitary Assay	196.19		
Ergot Assay	25.29		
Cod Liver Oil Reference Standard Assay	170.39	Dr. O. L. Kline[f]	35.00
Suture Absorbency Test	225.00		
Miscellaneous	47.58		
	$2,682.05		$3,470.37
			2,682.05
Total			$6,152.42

[a] Assistant to Chairman Jordan in revising proximate assays.

[b] Assistant to Chairman Krantz in checking tests and assays of inorganic chemicals. New Heavy Metals tests have been developed and tried on all inorganic as well as organic chemicals.

[c] Assistants for Chairman Beal, Miss Sackter replacing Mrs. Johnson when the latter resigned. Assigned to check tests and assays and develop new monographs for organic chemicals at Dr. Beal's personal direction. Most of the new admissions to the Pharmacopœia fall to this Sub-Committee.

[d] Assistant to Chairman Jenkins in checking new molecular weights.

[e] Assistant in the study of problems relating to the preservation of Digitalis.

[f] Assistant for compilation of Reference Cod Liver Oil standardization data for publication.